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A Journal of Pulmonary Diseases

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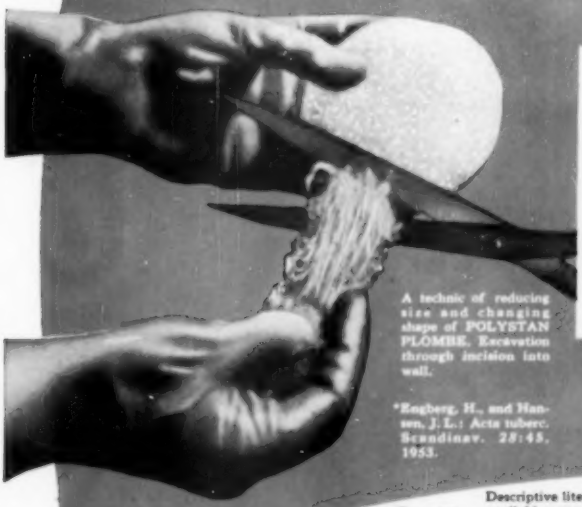

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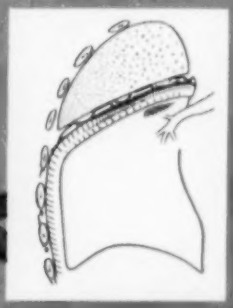

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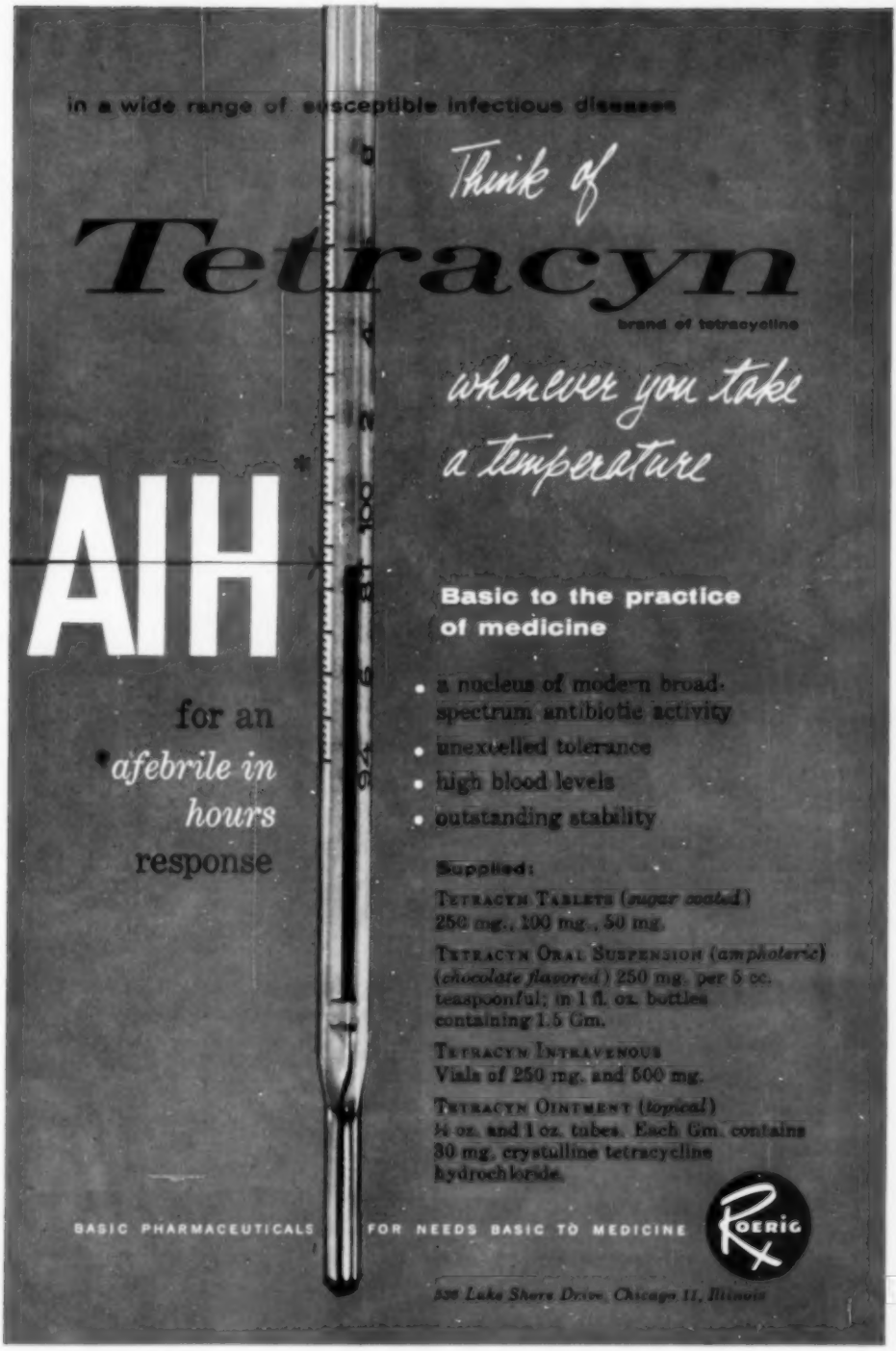
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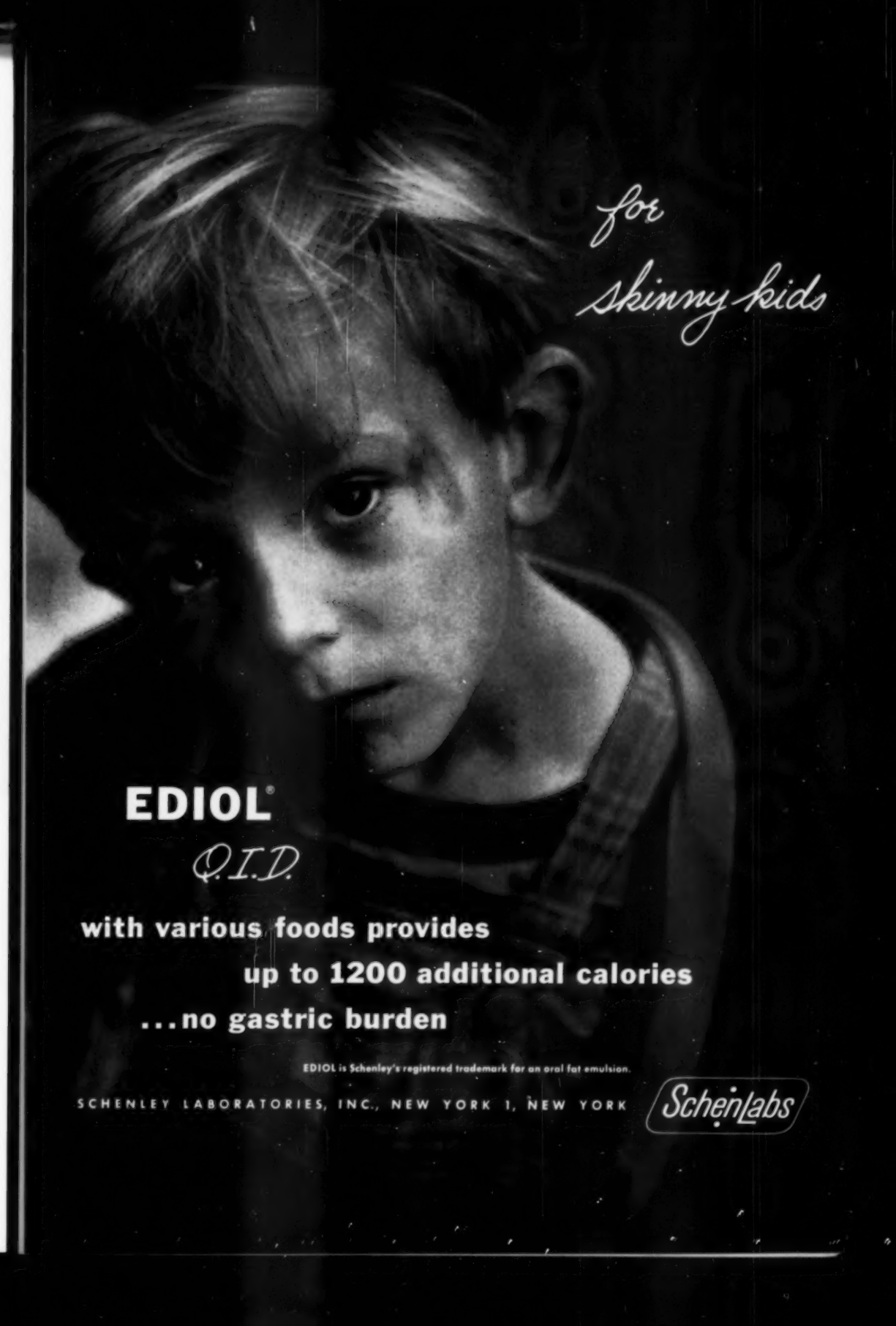
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BRONCHOGRAPHIC STUDIES IN BRONCHIECTASIS BEFORE AND AFTER PULMONARY RESECTION[1]

J. ANTRIM CRELLIN, J. STAUFFER LEHMAN, DANIEL MASON, AND JOSEPH L. CURRY

(Received for publication September 30, 1953)

INTRODUCTION

Well-established bronchiectasis is an irreversible bronchopulmonary disease. It is well known that surgical resection of diseased pulmonary segments or lobes offers the only hope for cure of the affliction. The end results of such pulmonary resections are not invariably satisfactory, however, nor is complete cure always effected. This may be due to inability or inadvisability of complete removal of all diseased bronchopulmonary segments. Nevertheless, occasional observations have convinced the writers that bronchiectasis can become manifest some time after surgery in segments of lung preoperatively free of evidence of bronchiectasis, in so far as can be determined by present diagnostic methods.

For the purpose of obtaining further information concerning the incidence of postoperative reappearance of bronchiectasis following surgical resection, a study was made of cases of bronchiectasis evaluated by Lipiodol bronchograms both before and some time after pulmonary resective surgery. To date, such pre- and postoperative bronchograms have been obtained in 44 nonconsecutive cases. Special interest has been directed toward a study of what changes, if any, occurred in the remaining segmental bronchi following resective surgery.

METHOD OF STUDY

Five-lobe Lipiodol bronchograms were obtained as an essential part of the diagnostic work-up of each patient prior to resective surgery. The *transnasal endobronchial catheter* method was regularly employed in obtaining all of the bronchograms (1). Accurate mapping of the bronchial distribution in all five lobes has been considered essential to complete preoperative evaluation of the extent and degree of bronchiectasis. For purposes of orientation, illustrations of normal right and left bronchograms in postero-anterior, lateral, and oblique projections (figures 1 to 6) are included in this paper.

[1] From the Departments of Medicine and Radiology, the Hahnemann Medical College and Hospital, Philadelphia, Pennsylvania.

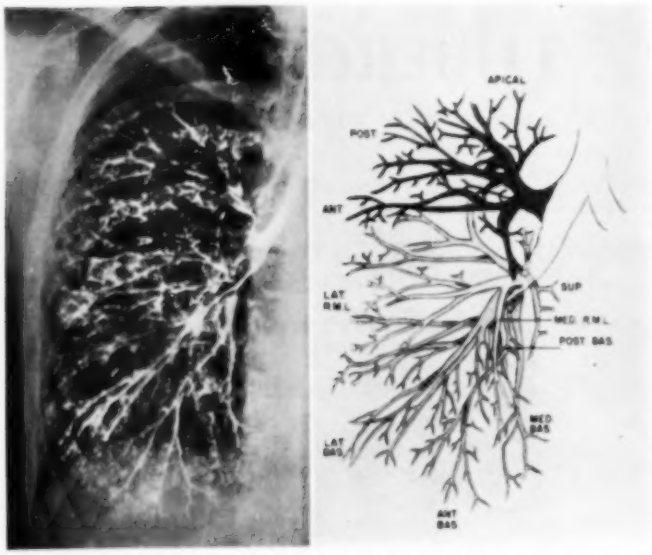

FIG. 1. Normal bronchogram, right lung: postero-anterior projection. Accompanying diagrammatic sketch shaded to delineate distribution of bronchi by lobes.

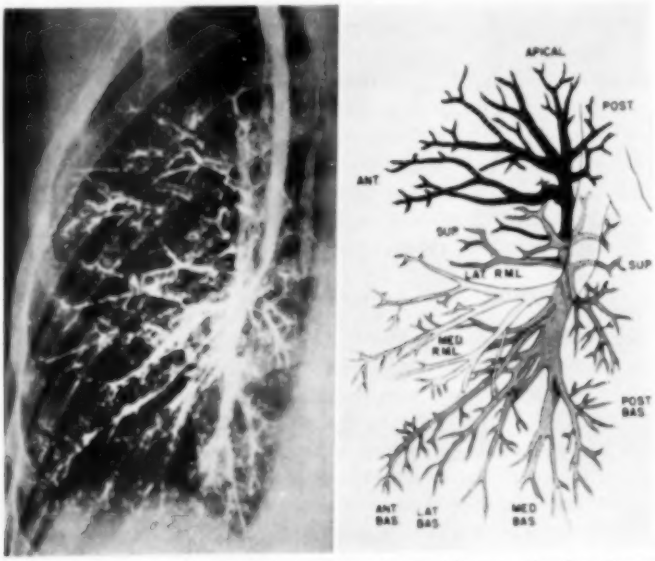

FIG. 2. Normal bronchogram, right lung: left anterior oblique projection. Accompanying diagrammatic sketch shaded to delineate distribution of bronchi by lobes.

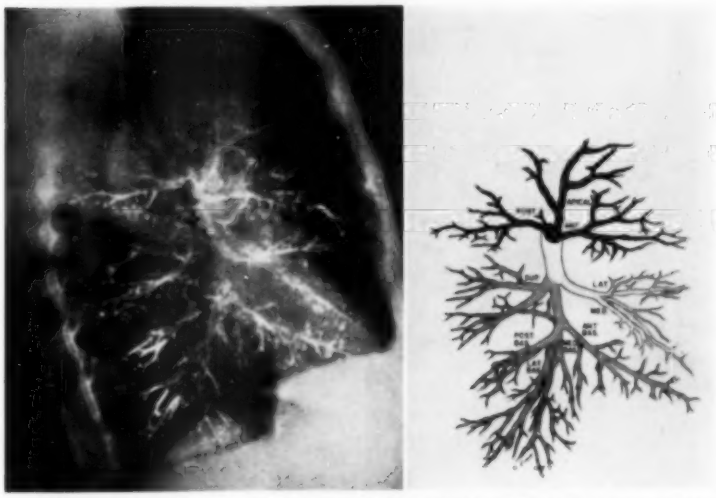

FIG. 3. Normal bronchogram, right lung: right lateral projection. Accompanying diagrammatic sketch shaded to delineate distribution of bronchi by lobes.

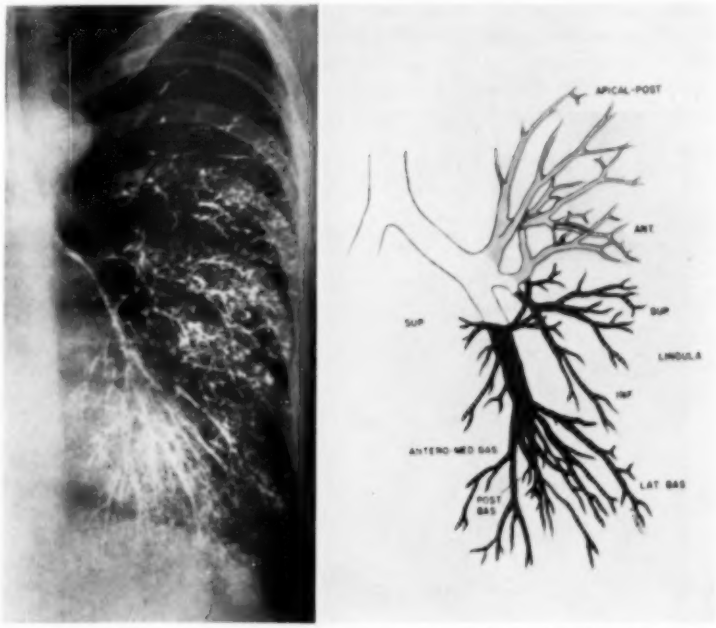

FIG. 4. Normal bronchogram, left lung: postero-anterior projection. Accompanying diagrammatic sketch shaded to delineate distribution of bronchi by lobes.

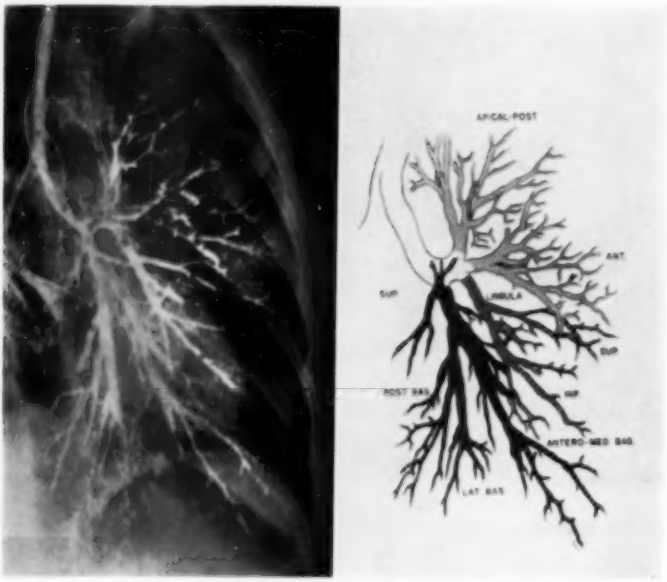

FIG. 5. Normal bronchogram, left lung: right anterior oblique projection. Accompanying diagrammatic sketch shaded to delineate distribution of bronchi by lobes.

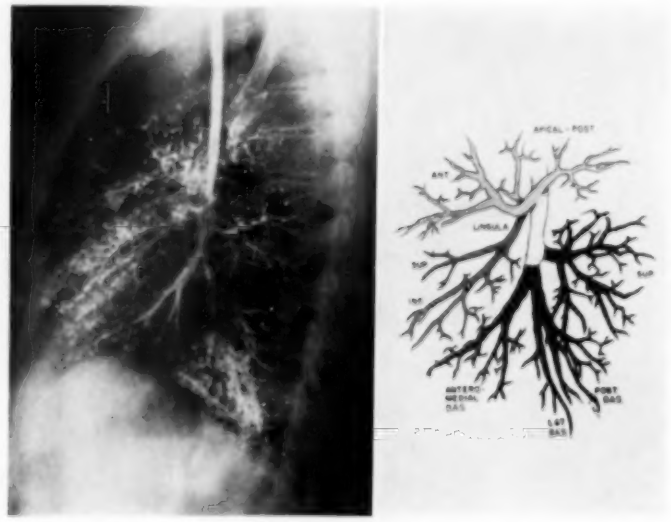

FIG. 6. Normal bronchogram, left lung: left lateral projection. Accompanying diagrammatic sketch shaded to delineate distribution of bronchi by lobes.

At some time subsequent to resective surgery, postoperative bronchograms were obtained. The shortest time interval between operation and postoperative bronchography was five months; the longest, two years.

OBSERVATIONS

The comparison of pre- and postoperative bronchograms has demonstrated that the degree of rearrangement of the bronchial tree and displacement of the remaining bronchi following resection of bronchopulmonary segments or lobes was always in direct proportion to the volume of lung removed. This observation is demonstrated in figure 7, in which minimal bronchial displacement is shown following removal of the lingula. This stands in contrast to the findings shown in figure 8, which reveals marked displacement of the remaining bronchi following left lower lobe removal. When an upper lobe is removed, the remaining inferior lobe or lobes enlarge and rotate upward, illustrated in figure 9, which demonstrates the position of the right middle and lower lobe bronchi following right upper lobe resection.

Preoperative Findings

Of the 44 cases of bronchiectasis, 20 were in males and 24 in females. Nineteen of the 44 patients had bilateral bronchiectasis. On the basis of bronchographic studies, the left lung was involved in 36 patients and the right lung in 27. Of the 36 cases of left lung bronchiectasis, 21 (58.3 per cent) showed combined left lower lobe and lingular involvement. Of the 27 cases of right lung involvement, the combination of middle and right lower lobe bronchiectasis was present in 17 (63 per cent). Of the 25 cases of unilateral bronchiectasis, the left lung was involved in 17 and right lung in 8.

Postoperative Findings

Unilateral resective surgery was performed on the left lung in 26 patients and on the right lung in 10 patients. Eight patients had bilateral surgery. Twenty-five patients had some type of segmental resection.

Postoperative bronchograms revealed no evidence of residual or new bronchiectasis in the operated lung in the majority of cases (25, or 56.8 per cent). In the remaining 19 cases, there was either persistence or development of bronchiectasis in the operated lung. Of these 19 cases, 4 are explainable on the basis of incomplete removal of all diseased segments of the operated lung. In each of these cases, postoperative bronchograms revealed persistence of a pulmonary segment thought to have been removed by the surgeon (figure 10).

Postoperative bronchiectasis developed in 4 patients as the result of postoperative complications (empyema with bronchopleural fistula).

In 10 patients bronchiectasis was detected postoperatively in bronchopulmonary segments which had been considered to be normal on the basis of preoperative bronchograms. Figures 11 and 12 illustrate the occurrence of bronchiectasis in apparently previously nonectatic bronchopulmonary segments. It is to be noted that, of the 10 patients in whom postoperative bronchiectasis was found,

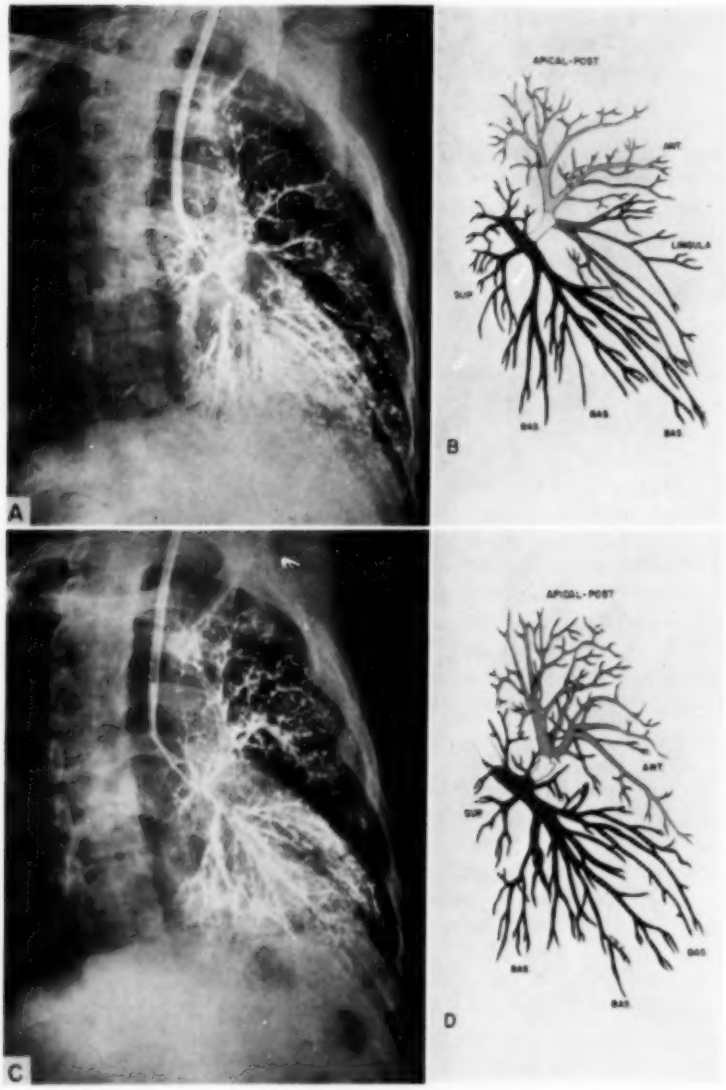

FIG. 7. Minimal rearrangement and displacement of bronchi following removal of only a small volume of lung (lingula): *A* and *B*. Preoperative bronchogram, left lung, right anterior oblique projection; *C* and *D*. Postoperative bronchogram, right anterior oblique projection, showing minimal displacement and rearrangement of upper and lower lobe bronchi following removal of the lingula.

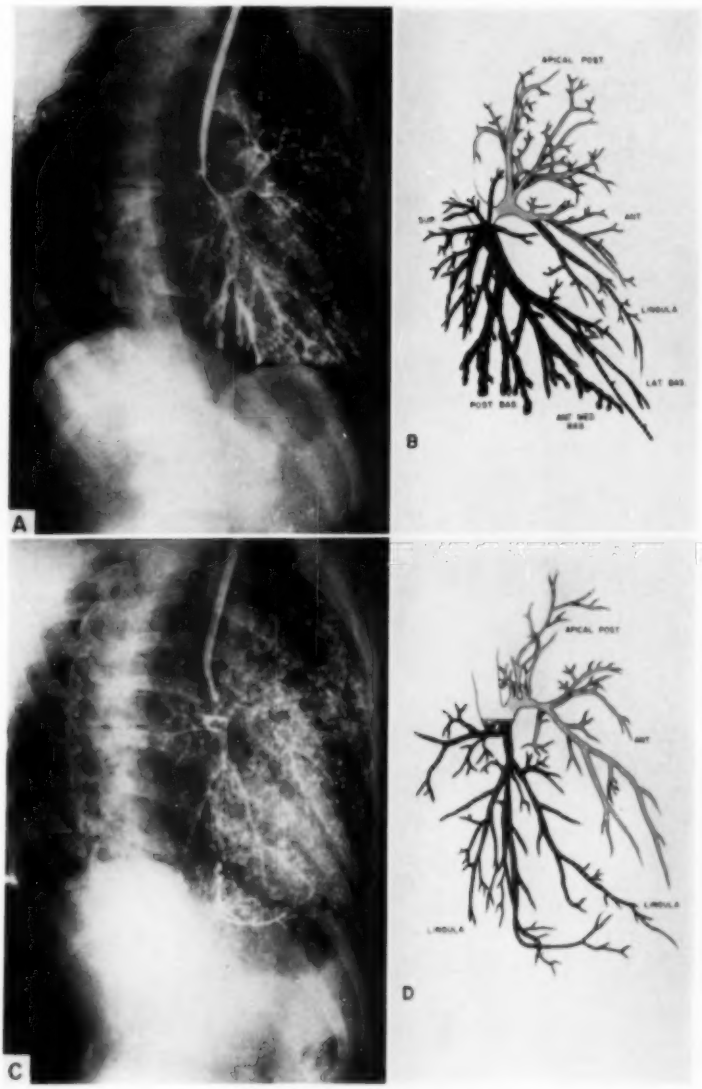

FIG. 8. Marked rearrangement and displacement of bronchi following removal of a large volume of lung (left lower lobe). *A* and *B*. Preoperative bronchogram, left lung, right anterior oblique projection; *C* and *D*. Postoperative bronchogram, right anterior oblique projection, showing marked displacement and rearrangement of remaining bronchi following removal of left lower lobe. Lingula rotated downward into position formerly occupied by left lower lobe.

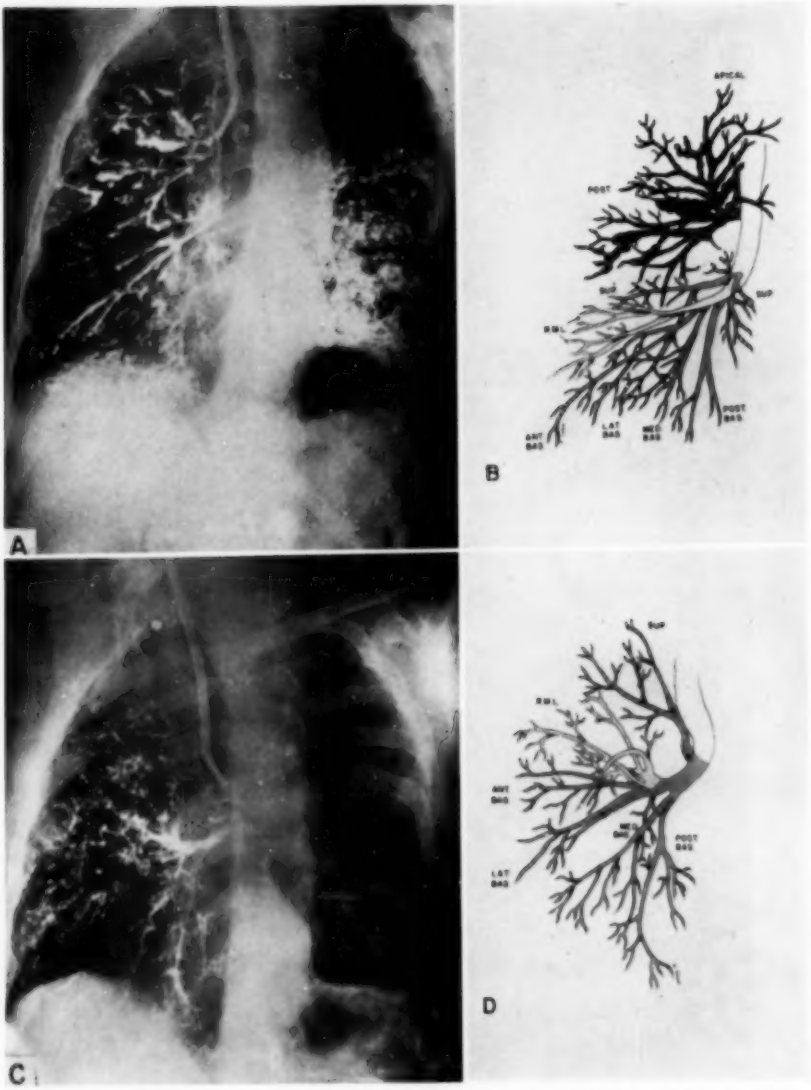

FIG. 9. Bronchial rearrangement after upper lobe removal (right upper lobe). *A* and *B*. Preoperative bronchogram, right lung, left anterior oblique projection; *C* and *D*. Postoperative bronchogram, left anterior oblique projection, showing upward displacement of the superior division of right lower lobe and the right middle lobe into the position formerly occupied by right upper lobe.

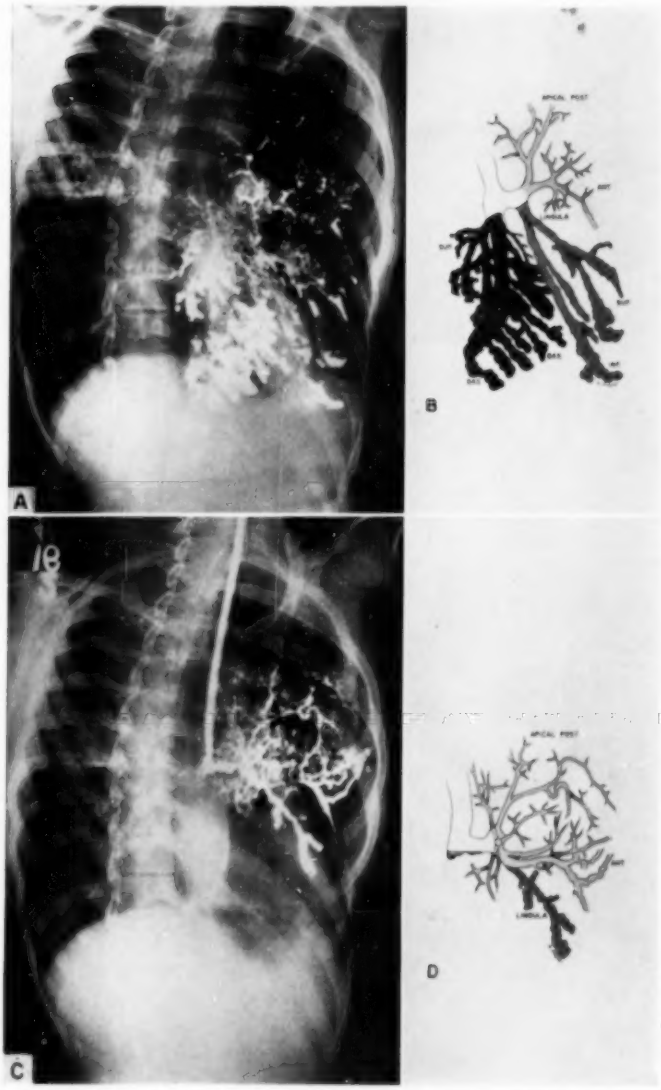

FIG. 10. Residual bronchiectasis as the result of incomplete removal of diseased segments. *A* and *B*. Preoperative bronchogram, left lung, right anterior oblique projection, showing saccular bronchiectasis, left lower lobe, lingula and anterior segment, left upper lobe; *C* and D. Postoperative bronchogram, left lung, right anterior oblique projection (eight months following stated lingulectomy and anterior segmental removal of left upper lobe and entire left lower lobe). Bronchiectasis in unremoved superior segment of lingula and anterior segment, left upper lobe.

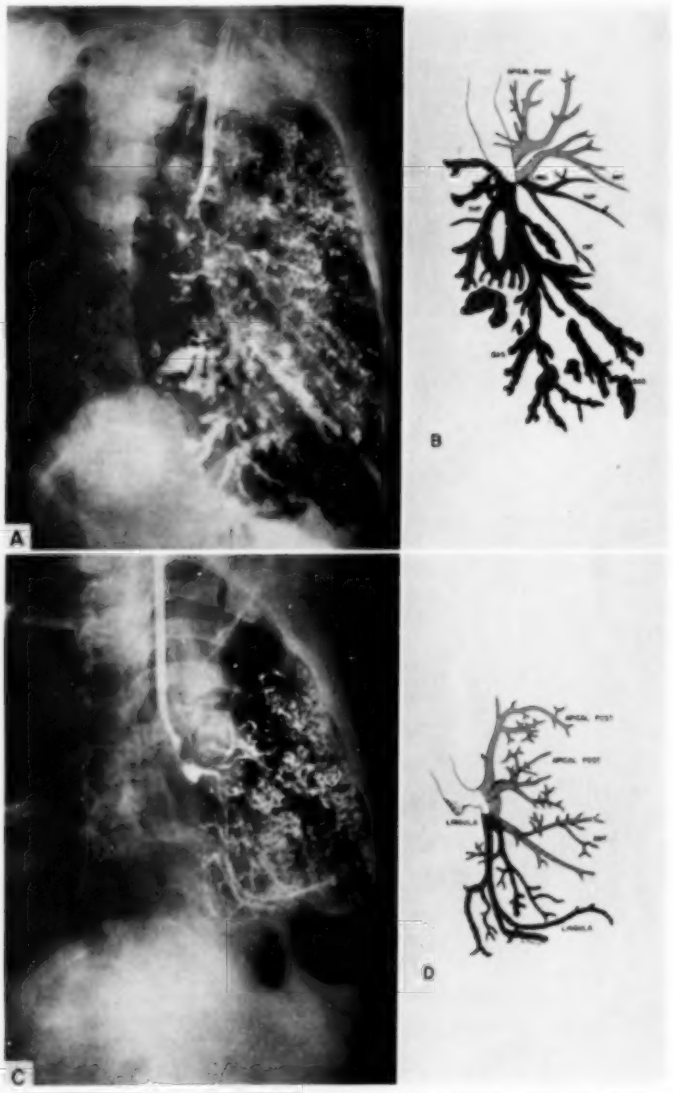

FIG. 11. Postoperative occurrence of bronchiectasis in pulmonary segments preoperatively evaluated as normal. *A* and *B*. Preoperative bronchogram, left lung, right anterior oblique projection, showing extensive saccular bronchiectasis of entire left lower lobe. Lingula and entire left upper lobe apparently uninvolved; *C* and D. Postoperative bronchogram, left lung, right anterior oblique projection (ten months following removal of left lower lobe). Lingula rotated into position formerly occupied by left lower lobe. Moderate cylindrical bronchiectasis of lingula.

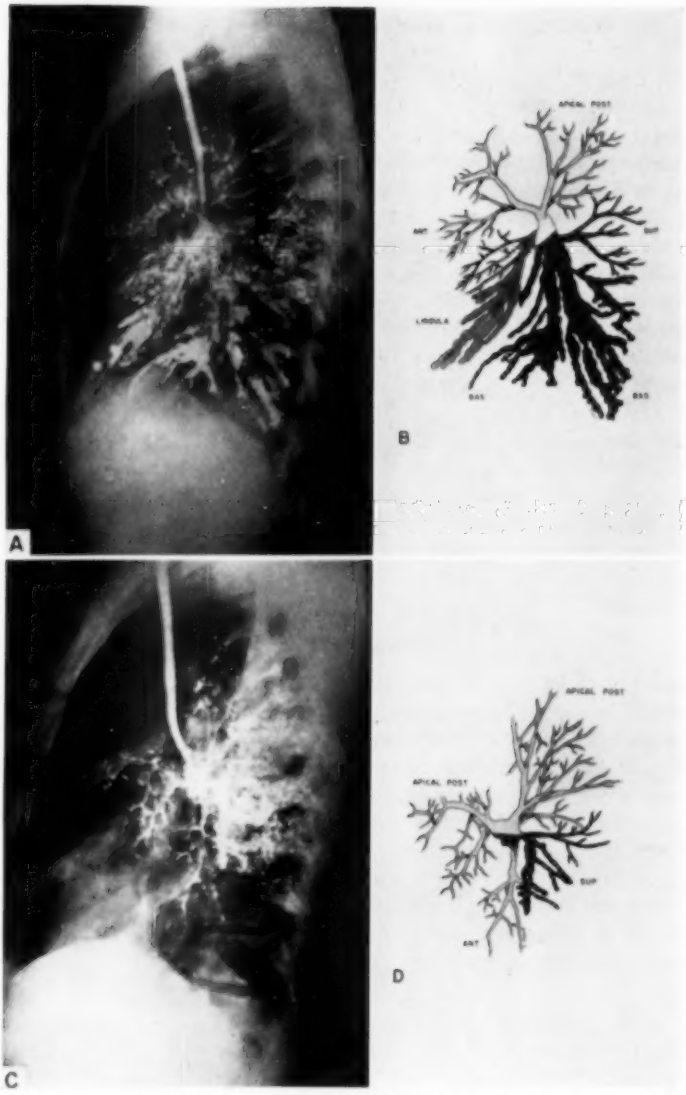

FIG. 12. Postoperative occurrence of bronchiectasis in a pulmonary segment preoperatively evaluated as normal. *A* and *B*. Preoperative bronchogram, left lung, left lateral projection, showing cylindrical and saccular bronchiectasis involving the lingula and left lower lobe. Superior segment of left lower lobe appears to be uninvolved; *C* and *D*. Postoperative bronchogram, left lung, left lateral projection (thirteen months following lingulectomy and removal of the inferior segment of left lower lobe). Anterior segment of left upper lobe rotated into position formerly occupied by inferior segment of left lower lobe. Inferior bronchi of remaining superior segment of left lower lobe ectatic and displaced downward.

6 had had segmental resections. Of these 6 patients with segmental resections, 5 had bilateral disease; 3 of these 5 had bilateral surgery, 2 had only unilateral surgery. In cases of bilateral disease and unilateral surgery, emphasis must be placed upon the ectasia in the contralateral lung as a possible focus to "feed" infection to the remaining portions of the operated lung. It was noted that 2 of the patients with bilateral disease and segmental resections had only unilateral surgery. Even in those cases of bilateral surgery, there was a time interval between operations for each lung, during which the possible factor of focal "feeding" might have been operative in promoting postoperative bronchiectasis. Of the 4 patients with nonsegmental resections who developed postoperative bronchiectasis in areas previously considered free of disease, 2 had bilateral ectasia, and both of these patients had bilateral surgery.

In all of the 10 cases (postoperative bronchiectasis in previously unaffected segments) there was a variable degree of distortion and displacement of the remaining bronchi. It is noteworthy that, of these 10 patients with postoperative bronchiectasis, 6 had bronchiectasis of moderate to marked degree, and one of minimal degree, on the nonoperated side (total of 7 cases). In 6 of the patients the postoperative bronchiectasis developed on the operated side. In one patient who had bilateral surgery, the postoperative ectasia developed on the side last operated upon.

Clinical Results

Clinical results in these 44 patients, as adjudged by gain in weight, sense of well-being, diminution in cough and amount of sputum, and number of subsequent respiratory infections, are tabulated in table 1.

Of the 10 patients who developed postoperative bronchiectasis (excluding those with incomplete removal of diseased segments or postoperative complications), 6 had segmental resections with results considered excellent in 3, good in 2, and poor in one. In the nonsegmental cases (4 of the above-described 10), the clinical results were excellent in 3 and poor in one.

The one patient with a poor result deserves special mention because of the time factor as far as clinical evaluation is concerned. At one year after operation the result was classified as "excellent," whereas at two years it was classified as "poor."

The patient was a 19-year-old girl with extensive bilateral bronchiectasis which involved the right middle and right lower lobes, as well as the lingula and left lower lobe. At the first

TABLE 1

CLINICAL RESULTS IN 44 NONCONSECUTIVE CASES

RESULT OBTAINED	NUMBER OF CASES
Excellent	26
Good	13
Fair	1
Poor	4

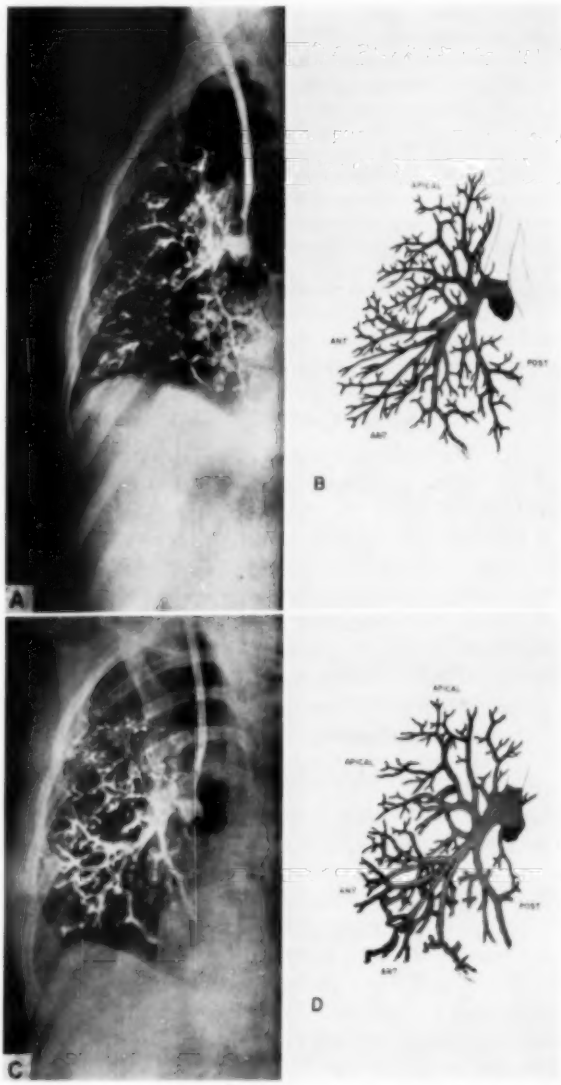

FIG. 13. Postoperative occurrence of bronchiectasis in an upper lobe. *A* and *B*. Postoperative bronchogram, right lung, left anterior oblique projection. Marked displacement and rearrangement phenomena of right upper lobe bronchi observed one year following removal of right middle and right lower lobes. No bronchiectasis evident in right upper lobe; *C* and *D*. Further bronchogram, right lung, two years following removal of right middle and right lower lobes. Evidence of cylindrical bronchiectasis involving the distended right upper lobe, particularly evident in bronchi presenting greatest degree of rotation and displacement.

operation the right middle and right lower lobes were removed, following which she had a rather stormy postoperative course, due to retention of secretions.

The left side was operated upon four months later, at which time the left lower lobe was removed without difficulty. Removal of the lingula was abandoned because of sudden pulmonary edema. At the time of the second operation, the lingular artery had been ligated. One year postoperatively, a bronchogram showed the remaining bronchial segments of the right upper lobe to be normal. The remaining portion of the left lung was normal, with the exception of the lingular bronchi, which showed further saccular dilatation. She was classified as being 90 per cent improved (excellent result) at this time. A bronchogram was repeated two years postoperatively, at which time the left upper lobe and lingula remained the same, but the segments of the right upper lobe revealed extensive cylindrical bronchiectasis. Eighteen months postoperatively the patient experienced a respiratory infection, following which there was a persistent increase in cough and sputum.

The result obtained in this patient was reclassified as "poor" at the end of the second postoperative year (figure 13).

DISCUSSION

The present series of 44 cases is an inadequate basis for any conclusive statistical analysis of the incidence of persistence or development of postoperative bronchiectasis. A discussion of "postoperative bronchiectasis" naturally raises the question of the basic pathogenesis of bronchiectasis. The present study was not planned to investigate the basic pathogenesis of bronchial ectasia nor does it throw any light on the underlying cause or causes of this disease. The study was directed rather to a determination of the frequency of residual or recurrent bronchiectasis after surgical treatment of the affliction. Admittedly, it is difficult to evaluate the several factors which may be of significance in the postoperative development of bronchiectasis in segments of lung preoperatively evaluated as free of disease. It seems appropriate, however, to discuss some of the possible causes for such postoperative appearance of bronchiectasis which have suggested themselves to the writers.

There may have been preoperative bronchiectasis present which was not demonstrated by present bronchographic procedures. Stewart and Kennedy (2), in their excellent paper, ask: "Can the distribution of the disease be accurately localized to bronchopulmonary segments by means of the combined evidence of bronchography and gross pathological findings at the operating table, and is a single bronchopulmonary segment, which may itself become over-distended in its role as a space filler, a liability or an asset to the patient?"

Such a question poses a consideration of the accuracy of bronchographic identification of minimal or minor degrees of bronchial dilatation. As with all roentgenographic procedures, it is reasonable to assume that quite small changes in bronchial lumen diameter may not be definable. There may well be, in certain instances, minimal ectatic changes which defy recognition either by bronchography or by gross visual inspection. The finite limits of bronchographic diagnostic accuracy are probably undefinable.

The rearrangement distribution and displacement phenomenon, particularly the dependent position of bronchi rotated to a basilar portion of the hemithorax

plus their frequent tortuous course, could enhance the possibility of bronchopulmonary suppurative pneumonitis, should the proper infection occur. It is not illogical to assume that there might be serious interference with bronchial drainage. It does not seem reasonable to assume that following pulmonary resection the remaining bronchi, if displaced or tortuous or both, would drain as adequately as if they were in their normal position. There are other factors besides distortion and displacement also to be considered, e.g., unrecognized ectasia in areas of lung preoperatively evaluated as nonectatic; and feeding from the opposite lung in bilateral bronchiectasis. However, while the writers have no supporting experimental observations, they incline to the belief that the phenomenon of bronchial distortion and displacement and the frequent tortuous course of rearranged bronchi which follows pulmonary resection is at least one of the factors promoting the development of postoperative bronchiectasis.

In bilateral bronchiectasis the focus of infection in the nonoperated lung feeding infection to the operated lung may be a significant factor. Bronchiectasis is not infrequently a unilateral disease. (In the present series of 44 patients, 25 had unilateral disease as judged by what were considered to be satisfactory bronchographic studies.) Whatever may be the exact pathogenesis of bronchiectasis, it seems unlikely that a focus of infection (bronchiectasis) in one area of lung is the sole responsible agent for the spread of bronchiectasis to other areas of lung, particularly to the opposite lung. The writers have no information concerning how great a factor bronchiectasis in a contralateral lung may be in the promotion of bronchiectasis in a lung from which all of its present ectasia has been removed. While admitting that "feeding" from an involved contralateral lung may be one of the factors concerned in the development of such "postoperative bronchiectasis," it is highly doubtful that it is the most significant factor. If such "feeding" alone were the most significant factor, one might expect to find bronchiectasis regularly a bilateral disease.

The possible influence of segmental resection deserves consideration. In certain instances in which all but a single segment of a lobe has been removed, the bronchi of the remaining segment assume an extreme degree of tortuosity, distortion, and angulation. On some occasions the writers have observed such distorted and displaced bronchi to become ectatic. Just what factors account for the appearance of ectasia in residual segments of an operated lobe is conjectural. All of the factors mentioned in the foregoing discussion may well be involved.

The length of time that any of the above-mentioned factors exist may prove to be the most significant influence on the development of postoperative bronchiectasis.

SUMMARY

An analysis of the pre- and postoperative bronchographic findings in 44 nonconsecutive cases of bronchiectasis has been presented.

The extent and degree of rearrangement of the bronchial tree and displacement of the remaining bronchi following resection of bronchopulmonary segments or lobes were always in direct proportion to the volume of lung removed.

In 25 of the 44 cases, there was no evidence of residual or recurrent bronchiectasis in postoperative bronchograms.

In 19 cases there was either postoperative persistence or development of bronchiectasis in the operated lung. Nine of these cases are explainable on the basis of incomplete resection or postoperative complications. Ten patients developed bronchiectasis postoperatively in areas of lung which had been considered to be free of disease on evaluation before operation.

The several factors considered to be of possible significance in the postoperative development of bronchiectasis have been discussed.

SUMARIO

Estudios Broncográficos en la Bronquiectasia Antes y Después de la Resección Pulmonar

Preséntase un análisis de los hallazgos broncográficos pre- y postoperatorios en 44 casos no consecutivos de bronquiectasia.

La extensión y la intensidad de la redisposición del árbol bronquial y el desplazamiento de los bronquios restantes después de la resección de segmentos broncopulmonares o lóbulos guardaron siempre proporción directa con el volumen de pulmón extirpado.

Veinticinco de los 44 casos no presentaron signos de bronquiectasis residual o recurrente en los broncogramas postoperatorios.

Diecinueve casos revelaron ya persistencia o formación postoperatoria de bronquiectasia en el pulmón operado. Nueve casos tienen explicación a base de resección incompleta o complicaciones postoperatorias. Diez casos presentaron formación postoperatoria de bronquiectasia en zonas pulmonares consideradas preoperatoriamente como exentas de enfermedad.

RESUME

Etudes bronchographiques dans la bronchiectasie avant et après la résection pulmonaire

Une analyse de bronchogrammes pré et post-opératoires dans 44 cas non consécutifs de bronchiectasie est présentée.

L'étendue et la degré des modifications de l'arbre bronchique et le déplacement des bronches restantes, après résection de segments broncho-pulmonaires ou de lobes, ont toujours été directement proportionnels au volume du tissu pulmonaire réséqué.

Sur 44 cas, 25 n'ont présenté aucun signe de bronchiectasie résiduelle ou récidivante dans les bronchogrammes post-opératoires.

Les 19 autres cas montraient la persistance ou le développement de la bronchiectasie dans le poumon opéré. Dans 9 cas, ces conditions étaient imputables à une résection incomplète ou à des complications post-opératoires. Dans 10 cas, on a observé le développement de la bronchiectasie dans des aires pulmonaires ayant été considérées indemnes de cette lésion avant l'intervention.

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MEDICAL MANAGEMENT OF ACUTE LUNG ABSCESS[1]

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INTRODUCTION

The medical treatment of acute lung abscess prior to the advent of antimicrobial therapy was unrewarding to a great extent for patient and physician alike. In a large number of the cases, as high as 40 to 60 per cent in some reported series, the process lapsed into chronicity, became associated with complications, and became a surgical problem. The reversal of this type of result on the chest service of the Harlem Hospital, New York City, where a number of patients with acute lung abscess are seen each year, has prompted a report of the experiences on a series of patients treated between the period April, 1949, to April, 1952.

It is believed that the results observed are of considerable interest, for Brock (1), as late as 1948, reported the conclusion that acute lung abscess was an essentially surgical disease with medical management only to be used as an expectant temporization. Moreover, Sweet (2), as recently as 1950, expressed the opinion that surgery should be performed early.

PLAN OF INVESTIGATION

The present material consists of 37 cases which meet the criteria of acute uncomplicated lung abscess clinically and roentgenographically. All patients were admitted to the hospital within two to ten days of the onset of their illness. The classical complaints were fever, chest pain, and cough productive of foul sputum. The predisposing causes were dental extractions or exposure to cold, following either submersion or drunkenness. In all of the cases the diagnosis was suspected clinically when the patients were first seen, either because of history, foul sputum, clubbing, acute illness with definite anemia, physical signs, or the invariable presence of roentgenographic evidence of cavity with fluid level.

Stivelman and Kavee (3) in 1946 reported a series of cases of putrid lung abscess treated with penicillin. The present patients were treated with penicillin, penicillin with sulfadiazine, chlortetracycline (Aureomycin), or oxytetracycline (Terramycin). If the results seemed slow or inadequate, various combinations of these drugs were used.

When oxytetracycline became available, the response of very critical patients treated with this drug was so spectacular and impressive that it seemed that this would be the superior drug for the treatment of uncomplicated lung abscess. An analysis of the material, however, resulted in the conclusion that no one of the above drugs is alone superior. Each drug used in adequate dosage alone or in combination gives comparatively satisfactory results.

[1] From the Department of Medicine, Harlem Hospital, New York City Department of Hospitals.

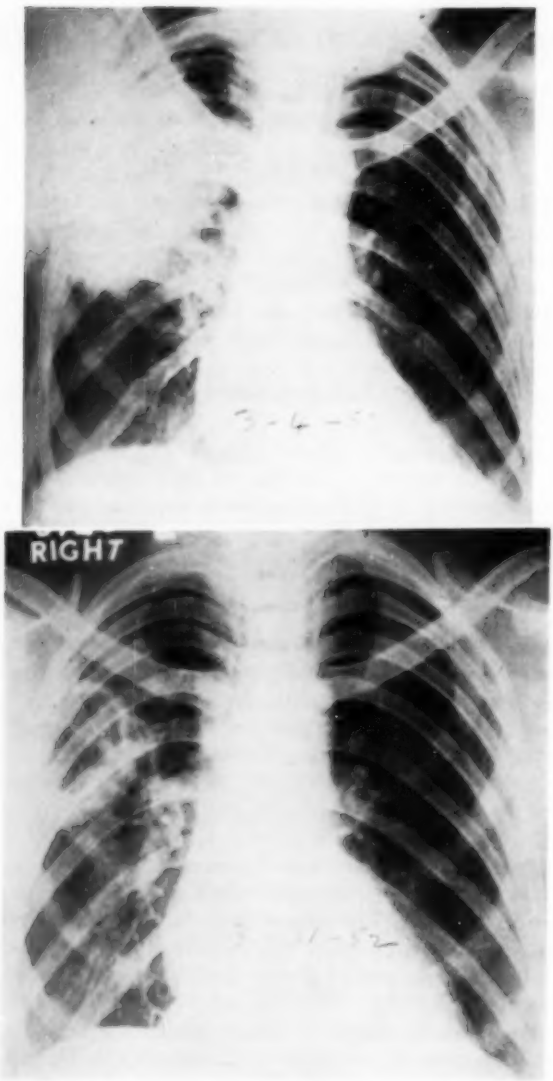

FIG. 1 (Upper). Showing an area of pneumonitis, simulating a lobar pneumonia, in the right upper lung field on March 4, 1952, the admission date of the patient.

FIG. 2 (Lower). Taken 27 days later, showing a large cavity with an area of surrounding pneumonitis at the site of the previous consolidation.

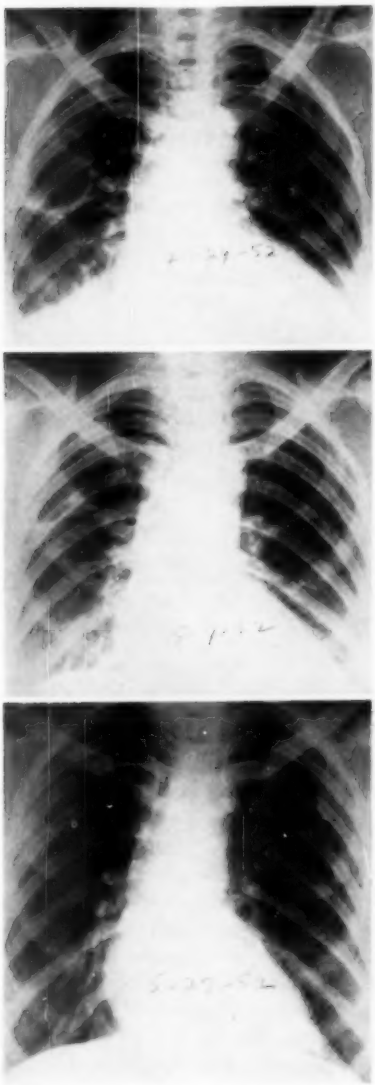

FIG. 3 (Upper). Taken on April 29, 1952, showing that most of the perifocal pneumonitis has cleared and a large cavity remains.

FIG. 4 (Center). Showing the cavity to be much smaller; fibrosis, indicating healing, is present at the site of the lesion.

FIG. 5 (Lower). Chest film taken on May 27, 1952, eighty-four days after admission, reveals complete healing of the lung abscess with a residual fibrous scar in the second right anterior interspace.

TABLE 1

PENICILLIN AND SULFADIAZINE

NAME	SEX	AGE	LOCATION OF ABSCESS	CLINICAL CURE TIME IN DAYS	TOTAL CURE TIME IN DAYS
H. G.	M	50	Left upper lobe	21	55
C. F.	M	40	Right upper lobe	22	107
J. T.	M	32	Right upper lobe	19	31
J. R.	M	39	Right upper lobe	35	112
V. M.	M	42	Entire left lung	21	87
C. J.	M	36	Left upper lobe, right parahilar area	15	74
O. M.	M	37	Right middle and lower lobes	38	65
T. R.	M	55	Right base	24	54
W. T.	M	49	Right upper lobe	30	50
J. H.	M	38	Right upper lobe	18	42

TABLE 2

PENICILLIN

NAME	SEX	AGE	LOCATION OF ABSCESS	CLINICAL CURE TIME IN DAYS	TOTAL CURE TIME IN DAYS
D. G.	M	30	Left upper lobe	10	22
F. W.	M	37	Right upper lobe	21	45
G. S.	M	42	Left upper lobe, lower portion of right upper lobe	28	38

The term *clinical cure time* is used to designate the minimum duration of therapy in days required to reverse all signs and symptoms of toxicity, to diminish sputum output to less than 30 ml. daily, and to produce the first perceptible roentgenographic evidence of clearing. *Total cure time* indicates the *clinical cure time* plus the time required to produce complete roentgenographic clearing with the exception of minor noninfectious fibrotic residues and other irreparable anatomic changes, such as emphysema or atelectasis due to permanent fibrosis.

The reproductions of chest films in figures 1, 2, 3, 4, and 5 show the roentgenographic changes treated and cured by antimicrobial therapy. These films are typical of the roentgenographic findings and course of the 37 patients treated and cured without surgical therapy.

In table 1 may be seen the data on the 10 patients who received penicillin with sulfadiazine. It is to be noted that, in 70 per cent of these patients, the lesion was in the right lung and that the average time for total cure was 67.7 days.

Table 2 relates to 3 patients treated with penicillin alone. It is believed that this number of patients is too small from which to draw definite conclusions. Nevertheless, the total cure time of an average of thirty-five days is gratifyingly short; approximately 50 per cent when compared with the other groups.

TABLE 3

CHLORTETRACYCLINE

NAME	SEX	AGE	LOCATION OF ABSCESS	CLINICAL CURE TIME IN DAYS	TOTAL CURE TIME IN DAYS
N. M.	M	65	Right upper lobe	22	54
O. R.	M	23	Right upper lobe and middle lobes	17	62
J. T.	M	31	Left lower lobe, right base	38	79
G. G.	M	52	Right lower lobe	27	49
J. M.	M	39	Left upper lobe	25	81
L. H.	F	49	Right lower lobe	20	88
J. D.	M	41	Right lower lobe	17	67
R. M.	M	47	Right midlung field	17	24
C. T.	M	44	Right lower lobe	27	49
M. W.	F	22	Left lower lobe	17	67
M. C.	M	40	Right middle lobe, left hilum	10	69
J. H.	M	46	Right middle lobe and right base	21	83

TABLE 4

OXYTETRACYCLINE

NAME	SEX	AGE	LOCATION OF ABSCESS	CLINICAL CURE TIME IN DAYS	TOTAL CURE TIME IN DAYS
M. D.	M	48	Dorsal segment of right lower lobe	22	83
L. M.	F	32	Right root and right upper lobe	33	82
G. R.	F	31	Posterior portion of right upper lobe	28	74
J. F.	M	43	Right upper lobe	14	60
E. G.	M	24	Left midlung field	35	51
C. P.	M	25	Left midlung field	8	64

In table 3 may be seen the results in 12 patients who received chlortetracycline. Approximately 70 per cent of the lesions in these 12 patients occurred in the right lung alone. The average total cure time was 64.3 days.

In table 4 may be seen the results attained in 6 patients who received oxytetracycline. Almost 70 per cent of the lesions were in the right lung, with an average total cure time of 69 days.

The patients listed in table 5 represent a group in which the average total cure time was longer. This was to have been expected, for these 4 cases represent instances in which treatment with penicillin gave a poor response and chlortetracycline was substituted. The average total cure time was 95 days.

Results in 2 patients with left lung lesions which attained average total cure may be seen in table 6. These patients should have been in the oxytetracycline series but were inadvertently started on penicillin.

TABLE 5

SUMMARY OF FOUR CASES STARTED WITH PENICILLIN, COMPLETED WITH CHLORTETRACYCLINE

NAME	SEX	AGE	LOCATION OF ABSCESS	DURATION OF PENICILLIN THERAPY IN DAYS	CLINICAL CURE TIME IN DAYS	TOTAL CURE TIME IN DAYS
P. A.	M	41	Right upper lobe	110	124	152
T. C.	M	39	Entire right lung field	35	24	67
C. R.	M	43	Right lower lobe	42	52	91
J. S.	M	47	Right upper lobe	14	20	70

TABLE 6

SUMMARY OF TWO CASES STARTED WITH PENICILLIN, COMPLETED WITH OXYTETRACYCLINE

NAME	SEX	AGE	LOCATION OF ABSCESS	DURATION OF PENICILLIN THERAPY IN DAYS	CLINICAL CURE TIME IN DAYS	TOTAL CURE TIME IN DAYS
E. T.	F	30	Apex of left lower lobe	2	27	35
C. P.	M	56	Left upper lobe	15	25	94

The dosage schedule employed for the various drugs in the average case was as follows:

Penicillin—600,000 units intramuscularly every six hours until *clinical cure time*, then twice daily to *total cure time*

Sulfadiazine—1 gm. four times daily, orally

Chlortetracycline—250 mg. by mouth every six hours through *clinical cure time*; then reduced to three times daily through *total cure time*

Oxytetracycline—250 mg. by mouth every six hours through *clinical cure time*; then reduced to three times daily through *total cure time*

In 2 of the patients who were very ill, it was necessary, for a few days, to give oxytetracycline, 500 mg. intravenously in infusions of 5 per cent glucose, in conjunction with the oral treatment to combat the overwhelming toxicity caused by the abscess. Blood transfusions were given whenever the erythrocyte count was below three million.

DISCUSSION

The management and the results encountered in the series of 37 cases of lung abscess are presented in tables 1 through 6. In all of these cases, definite improvement was noted early in the course of medical treatment. Thus, with continued optimal medical management, complications failed to occur, clinical cures were attained, and resort to surgical procedures obviated.

In most of the cases, evidence of clinical cure time, as indicated by loss of fever, reduction in pulse rate and sputum volume, clearing of foul odor of expectoration, disappearance of the toxic, severely anemic and anxious state, was ob-

served between the eighth and thirty-eighth days. Desire for food and an interest in surroundings began to return. The patient could be permitted self-help and bathroom privileges. In a few cases in which response tended to be slow, the addition of one of the other drugs or a change to another antimicrobial brought about the desired benefit.

A further period of treatment beyond the average twenty-four-day clinical cure time was necessary in order to attain roentgenographic evidence of complete healing of the lung abscess. This stage is considered the total cure time and was attained in a maximum of one hundred and twelve days and within an average time of approximately sixty days. During this period most patients enjoyed an increasing degree of physical activity, and their chest films showed progressive healing of the abscessed lung areas. There has been no recurrence of pulmonary suppuration and no need for surgical intervention.

The results in the present series indicate that no dramatic response can be expected from either chlortetracycline or oxytetracycline in the treatment of acute lung abscess. In most cases penicillin seems to be just as effective provided dosage schedules are strictly adhered to, therapy is prolonged sufficiently, and due attention is given to supportive treatment, good nursing care, postural drainage, and bronchoscopy when indicated. Sulfadiazine as an adjunct to penicillin is desirable when not specifically contraindicated, but is not mandatory. The challenging effectiveness of penicillin perhaps relates to the usual pathogens likely to be encountered in acute lung abscess, i.e., the predominantly gram-positive oropharyngeal flora and the spirochetal organisms of the oral cavity.

Chlortetracycline and oxytetracycline, because of their greater antimicrobial range, do seem to succeed when penicillin occasionally fails, but even here one must not be led to quick conclusions. It may be that penicillin had laid the tedious groundwork for a relatively easy victory by the tetracycline drugs. The administration of these drugs by the oral route is of distinct advantage, but this must be weighed against their greater cost and the possible danger of emergence of pathogenic yeasts following the excessively thorough bacterial elimination they produce. Disturbances of normal body flora must also be taken into account when prolonged administration is contemplated. Vitamin K deficiencies which may follow destruction of the intestinal flora by chlortetracycline and oxytetracycline may possibly cause bleeding from abscess cavities, which are prone to bleed even in the absence of vitamin K deficiency.

All things considered, it is believed to be wisest to reserve drugs of the tetracycline series for unusually stubborn cases, in which cultures show that the causative organisms are generically or individually resistant to penicillin, or when penicillin has been given a fair trial and has failed to produce an adequate response. An alternate approach to this problem would be the development of a basic penicillin regimen interrupted by brief periods of tetracycline therapy, as suggested by Barach.

It is of interest to note that, during the period of the present study, the writers were also faced with the management of an additional 23 cases of suppurative

disease of the lung and/or its parietes which did not conform to the criteria of acute uncomplicated lung abscess. Some of these patients had had ineffectual thoracotomies for previous lung abscess; others had encapsulated empyema or pyopneumothorax. Still others had had previous chest drainage for empyema with complicating pneumonia. There can be no doubt that this type of suppurative thoracic disease—sometimes loosely and erroneously labeled lung abscess—is purely a surgical problem necessitating definitive nontemporizing surgery as soon as recognized. In these circumstances, the present writers assuredly subscribe to the use of all methods, enzymatic therapy, excision, antimicrobial therapy, and whatever combination of procedures will eradicate and evacuate all pus and diseased areas in the chest. Surgical aid was promptly sought for all of these patients.

It appears that the problems inherent in the treatment and management of lung abscess depend upon four principal considerations: First, whether the acute pulmonary abscess, putrid or nonputrid, is uncomplicated; second, whether there is complicating empyema at the onset; third, whether chronic suppurative disease of the lung is present; fourth, whether the lung abscess is a complication of coexisting pulmonary or other systemic disease.

SUMMARY

The medical management of 37 patients with lesions which conform to the criteria of acute uncomplicated lung abscess is presented.

The writers' experience with the use of penicillin, penicillin with sulfadiazine, chlortetracycline (Aureomycin), or oxytetracycline (Terramycin), and penicillin supplemented by chlortetracycline or oxytetracycline shows that in most cases good results were promptly obtained. In the few cases in which improvement appeared delayed under penicillin therapy, the resort to the tetracycline drugs provided a cure. Chlortetracycline and oxytetracycline seemed equally effective.

It is not believed that either drug offers a clear superiority, but that successful results depend upon prompt and adequate treatment in the majority of cases.

The availability of the broad-spectrum antimicrobial drugs has made it possible to cure acute lung abscess in a relatively short period of time without the need for surgery.

SUMARIO

Atención Médica del Absceso Pulmonar Agudo

Descríbese la asistencia médica de 37 casos que se conforman a las pautas establecidas para el absceso pulmonar agudo sin complicaciones.

Las observaciones de los AA. con el uso de penicilina, penicilina con sulfadiacina, clortetraciclina (Aureomicina) o oxitetraciclina (Terramicina) y penicilina complementada con clortetraciclina u oxitetraciclina demuestran que en la mayor parte de los casos se obtuvieron buenos resultados prontamente. En los pocos casos bajo penicilinoterapia en que pareció demorarse la mejoría, la utilización de las drogas micínicas logró la curación. La clortetraciclina y la oxitetraciclina parecen ser igualmente eficaces.

No se cree que una u otra droga posea superioridad absoluta, sino más bien que el éxito depende del tratamiento rápido y adecuado en la mayoría de los casos.

La disponibilidad de los antibióticos de espectro ancho y de las drogas quimioterapéu-

ticas ha hecho posible curar el absceso pulmonar agudo en un plazo relativamente corto sin necesidad de cirugía.

RESUME

Traitement médical de l'abcès aigu du poumon

Le traitement médical de 37 cas conformes aux critères de l'abcès aigu non compliqué du poumon est exposé. L'expérience acquise par les auteurs en utilisant la pénicilline, la pénicilline associée à la sulfadiazine; la chlortétracycline (Auréomycine) ou l'oxytétracycline (Terramycine) et avec la pénicilline plus la chlortétracycline ou l'oxytétracycline montre que dans la plupart des cas on obtient rapidement de bons résultats. Dans les rares cas où l'amélioration a été lente à se manifester avec la pénicilline, la guérison fut obtenue en faisant appel au groupe mycine. La chlortétracycline et l'oxytétracycline paraissent avoir la même efficacité.

Les auteurs ne pensent pas que l'un ou l'autre antibiotique soit doué d'une nette supériorité; ils estiment que dans la majorité des cas les résultats satisfaisants dépendent d'un traitement précoce et adéquat.

Les antibiotiques à spectre étendu et les préparations chimiothérapeutiques dont on dispose permettent de guérir l'abcès du poumon dans un temps relativement bref, sans recourir à la chirurgie.

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THE EFFECT OF ANTIMICROBIAL THERAPY ON THE PROGNOSIS OF PRIMARY TUBERCULOSIS IN CHILDREN[1, 2, 3]

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INTRODUCTION

Specific treatment of tuberculosis has now been in general use for approximately seven years and it seems wise at this time to assess its effect on the prognosis of primary tuberculosis and its complications. Before the era of antimicrobial therapy it was recognized that the mortality and morbidity of primary tuberculosis varied with many factors.

As tuberculosis is a contagious disease, the number of primary infections in children in any community reflects the amount of tuberculosis in the adult population. Thus, in areas where the tuberculosis rate is low, as in Denmark or in some parts of the United States (for example, Minnesota or Iowa), there is a low rate of infection in children and a very low death rate. On the other hand, children who live in poor, crowded sections of cities, where the tuberculosis rate is high, usually have more tuberculous infections and a higher death rate.

The prognosis of first infection tuberculosis, however, is not dependent solely on the extent and duration of exposure but is influenced by many social and economic factors, such as differences in diet and housing. Inherent resistance, about which relatively little is known, undoubtedly also plays a part in determining the outcome of the disease in any child. There are known geographic variations in the clinical picture of primary tuberculosis. Erythema nodosum, for instance, so frequently associated with tuberculosis in Scandinavian countries, is rarely seen in the United States.

Although it is not known whether such variations in the clinical picture have a direct influence on prognosis, it seems more accurate to attempt to evaluate the effect of antimicrobial therapy on prognosis by comparing groups of children in the same geographic area who come from similar social and economic levels.

Background of Investigation

The pediatric wards of Bellevue Hospital, a municipal hospital in New York City, have afforded the opportunity to study the prognosis of a group of tuberculous children who come mainly from very poor, overcrowded homes. In order to maintain as far as possible the similarity of the clinical material, only children with pulmonary

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[2] This study was aided by grants from the Committee on Medical Research of the American Trudeau Society, medical section of the National Tuberculosis Association; from the Division of Research Grants and Fellowships of the National Institutes of Health, Public Health Service; and from Parke, Davis and Company.

[3] Portions of this report were presented before the Seventh International Congress of Pediatrics, held at Havana, Cuba, on October 15, 1953.

primary tuberculosis were included in the study. Children with extrapulmonary primary tuberculosis or those with normal roentgenograms were excluded. Children whose roentgenograms showed calcification have not been included in the series since it is assumed that most of these children have already survived the most dangerous period of the disease.

In October, 1930, an intensive study was begun of every child with tuberculosis who entered the hospital and was shown to have primary pulmonary tuberculosis. Every child over one month of age received a Mantoux test, and a roentgenogram of the lungs was taken of every positive reactor. By 1947, when streptomycin therapy was introduced, a group of 980 children with primary pulmonary tuberculosis with no evidence of calcification had been assembled. These were consecutively diagnosed patients, ranging in age from three months through twelve years. Many of the children were admitted to the hospital for symptoms due to other diseases, and their tuberculosis was discovered incidentally. Approximately 85 per cent were found to be tuberculous either through use of the tuberculin test on admission or, if they were admitted to the hospital as tuberculous, had originally been diagnosed by case-finding methods, usually through a history of contact or by a tuberculin test.

The case fatality rate among these 980 children was high; 21.5 per cent of this group died as a direct result of primary tuberculosis; 9 of 10 of the deaths occurred within a year of the first diagnosis of tuberculosis. The highest death rates were in the youngest children; the lowest in the age group five through nine years of age.

Four conditions accounted for 95 per cent of the mortality: (*1*) meningitis, which caused 60 per cent of the deaths, (*2*) acute generalized miliary tuberculosis not associated with meningitis, which was responsible for an additional 10 per cent of deaths, (*3*) more protracted forms of generalized hematogenous tuberculosis, and (*4*) locally progressive primary tuberculosis with cavitation and bronchogenic spread.

OBSERVATIONS

Successful chemotherapy of tuberculosis in Bellevue Hospital began in 1944 with the use of a sulfone, Promizole[4]. This drug effected cures in some cases of miliary tuberculosis but failed to cure meningitis or other complications. Consequently there was no marked change in the mortality rate until the introduction of streptomycin in late December, 1946.

From 1947 through 1951, therapy with streptomycin, para-aminosalicylic acid (PAS), and the sulfone was available. During this five-year period, 421 children with primary pulmonary tuberculosis were admitted to Bellevue Hospital. As was true of the group prior to specific therapy, these 421 children were consecutive unselected cases.

In general, there had been little change in the type of patients. A high proportion of the children in both groups came from families of very low economic level, frequently receiving public assistance. The incidence of complications was about the same in both groups. The main differences between the two groups were the larger numbers of Negro children and of children under five years of age in the more recent group. Both of these factors might have been expected to show an unfavorable influence on prognosis.

Naturally, it would have been desirable to have had control groups in evalu-

[4] Supplied by Parke, Davis and Company (hereinafter designated *the sulfone*).

ating the effect of any agent on the prognosis of a disease. However, forms of disease which have a high fatality rate furnish the best clinical material for testing new means of therapy, and it was obviously impossible to withhold chemotherapy from children afflicted with such complications as tuberculous meningitis or miliary tuberculosis.

Both groups received essentially the same treatment aside from chemotherapy. All of the patients received the same type of diet and approximately equal amounts of bed rest.

The exact form of chemotherapy varied with the type of complication. Streptomycin and the sulfone were combined in the therapy of meningitis and of tuberculosis of obviously hematogenous origin, and streptomycin combined with PAS was the usual form of therapy in locally progressive primary tuberculosis.

Chemotherapy was not universally used but was given in only 35 per cent of the 421 patients. The use of specific therapy was considered essential only in children with the complications previously enumerated, which had produced 95 per cent of the mortality in the earlier group.

With this plan of treatment, the mortality from meningitis in the years 1947 through 1951 fell from 100 to 37 per cent. The death rate from all complications of primary tuberculosis fell from 21.5 to 5.0 per cent. In 1952, isoniazid was added to the therapeutic regimen, and during 1952–1953 the case fatality rate from primary pulmonary tuberculosis was lowered to 1.5 per cent and the death rate from meningitis to 12 per cent.

TABLE 1

DIMINISHING MORTALITY RATE FROM PRIMARY TUBERCULOSIS IN PATIENTS ON CHILDREN'S TUBERCULOSIS WARD OF BELLEVUE HOSPITAL FROM 1930 THROUGH 1953
(Decrease Is Apparently Related to Use of Specific Therapy*)

YEAR	NUMBER OF CASES	TREATMENT	MORTALITY
			per cent
1930–1946	980	No specific therapy	21.5
1947–1951	421	Streptomycin, PAS, and Promizole used in 35 per cent of cases	5.0
1952–1953	129	Isoniazid added	1.5

* One child with asymptomatic primary tuberculosis who died in a sickle-cell crisis is omitted from the mortality figures for 1947–1951; a death from acute laryngotracheobronchitis in primary tuberculosis without evidence of disseminated tuberculosis at autopsy is omitted from the 1952–1953 mortality figures.

DISCUSSION

From the viewpoint of mortality statistics, antimicrobial therapy has been effective in saving the lives of many children with serious complications of primary tuberculosis. Nevertheless, successful treatment should imply more than mere survival. The aim should be to restore the patient to a full and normal life.

It is important, therefore, to inquire into the condition of the children who have survived complications of primary tuberculosis which prior to chemotherapy were frequently fatal.

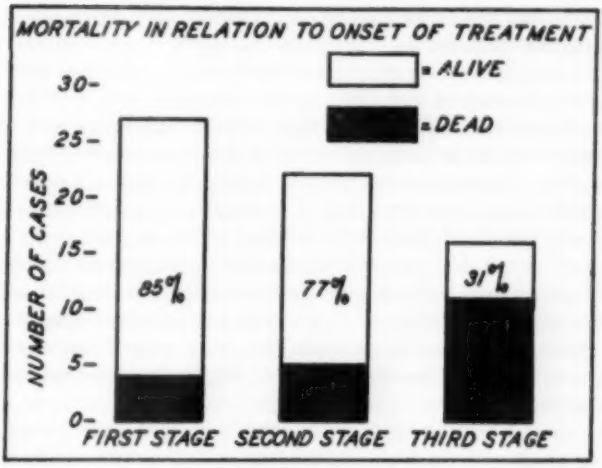

FIG. 1. Decrease in survival rate of tuberculous meningitis in relation to stage of meningitis during which treatment was instituted.

Of the 65 patients with meningitis treated during the years 1947 through 1953, 45 have survived; no relapses have occurred later than six weeks after discontinuing streptomycin. Most of the children are leading normal lives, usually after long periods of convalescence. Four children have residual hemipareses, and one a foot drop; only one of these patients required mechanical aid in locomotion. Diminution or loss of hearing has occurred in about one-third of the patients. Three survivors have severe behavior difficulties; all 3 had symptoms suggesting behavior problems before they had meningitis. Three infants are mentally deficient; several older children have low intelligence ratings, but their mental status prior to meningitis is unknown. Some children at first thought to be mentally deficient have improved steadily and now behave and respond to tests like normal children. Final evaluation of prognosis from a psychiatric and psychologic point of view should be made only after adequate periods of observation, perhaps as long as five years in some children.

The addition of isoniazid to the therapy of meningitis has already resulted in a higher survival rate. Recently the total dosage of streptomycin has been sharply reduced by giving streptomycin twice weekly after an initial daily course of four to six weeks. The use of streptomycin by the intrathecal route has been eliminated in recent cases. No deafness has occurred since these changes have been made.

Still newer and better antimicrobial agents will undoubtedly be developed but, because of the inherent nature of the disease, it seems unlikely that it will ever be possible to cure every case of tuberculous meningitis. The results of therapy of meningitis will always depend in part on the stage of the disease during which a diagnosis was established. Patients first treated in a late stage of the disease will not only have less chance of survival, but some who survive will inevitably show evidence of irreversible cortical damage. Therefore, as chemotherapeutic agents

improve, it will presumably be possible to save more lives at the cost of producing some defective children.

Results of treatment with isoniazid have been excellent in miliary tuberculosis, even when no streptomycin was added to the combined therapy of isoniazid and the sulfone. Moreover, isoniazid not only cures the hematogenous lesions but apparently prevents the development of clinical tuberculous meningitis.

The immediate prognosis of the other two complications of primary tuberculosis which were responsible for many of the deaths from primary tuberculosis has been greatly improved. Previously children with local progression of the primary focus with cavitation or with protracted hematogenous tuberculosis frequently died within six months of onset. Moreover, the survivors not infrequently experienced a relapse or developed new areas of tuberculous disease months or years later. The immediate deaths of children with these forms of tuberculosis can now be prevented and the disease can usually be rendered inactive by prolonged chemotherapy.

Following specific treatment many children show apparently complete regression of a tuberculous bronchopneumonia, with disappearance of the primary cavity on the roentgenograms and remission of all clinical symptoms. Such cases should not be termed cures, however, until a long period of observation after cessation of chemotherapy has failed to show roentgenographic or clinical evidence of relapse. In some of the Bellevue Hospital cases, surgery has been employed as an aid to chemotherapy, especially when cavitation could be demonstrated by planigram after a long course of treatment. Only further study of such cases will demonstrate whether surgery improves the prognosis of cases of cavitating primary tuberculosis treated by chemotherapy.

In the cases discussed so far, chemotherapy was used as a life-saving measure. Specific therapy was also used in another group of children with complications of primary tuberculosis of a less serious nature. In these cases streptomycin or, later, isoniazid, always in combination with PAS or the sulfone, was given on an elective basis in an attempt to alleviate symptoms, shorten the course of the disease, or improve the ultimate prognosis. The results of treatment have varied. Occasionally the results of antimicrobial therapy were definitive. For instance, the clinical picture of tuberculous laryngitis, a rare complication of primary tuberculosis, responded promptly to therapy. Likewise, cutaneous sinuses rapidly healed under specific therapy, often paving the way for treatment of the underlying tuberculous disease by surgery; thus, definitely improving the prognosis. Chemotherapy used for short periods before and after operative procedures on tuberculous patients has also had a favorable influence on prognosis since it enables the surgeon to remove with safety foci of active tuberculous disease from which future dissemination might have occurred. It has been difficult to evaluate the effect of chemotherapy on other complications, such as bone and lymph node tuberculosis, because the natural course of such complications is chronic and is often interspersed with periods of spontaneous remission. In still other complications, for example, endobronchial tuberculosis, it has seemed clear that chemotherapy has neither shortened the course of the disease nor alleviated symptoms.

Criteria for treatment of nonfatal complications of primary tuberculosis will obviously change as new drugs are introduced and as the results of previous chemotherapy on prognosis are re-evaluated after longer periods of follow-up observation.

In addition to the cases in which specific therapy was considered essential and those in which it was used on an elective basis, there remain a large number of patients with uncomplicated, largely asymptomatic primary tuberculosis who were not treated. These patients comprised about two-thirds of the ward population throughout the period studied from 1930 through 1953.

In these patients, streptomycin was not used for the following reasons. In the first place, no definite evidence has been obtained by any investigator that the resolution of the primary pulmonary focus is hastened by the use of streptomycin. In the second place, there is abundant evidence that complications in bones, nodes, and other areas develop and progress in patients under streptomycin therapy for other forms of tuberculosis, and many children developed meningitis while under intensive treatment for miliary tuberculosis. A third reason for withholding streptomycin in treatment of uncomplicated primary tuberculosis is the possibility, even with combined therapy, of the emergence of organisms resistant to streptomycin which might limit its later effective use; moreover, even in the absence of resistant organisms, there is evidence that retreatment is less effective.

The addition of isoniazid to the list of specific antituberculous agents may overcome the reluctance to use specific therapy in asymptomatic primary tuberculosis. There is as yet no clear evidence that the use of isoniazid hastens the resolution of the pulmonary primary focus. In another respect, however, isoniazid therapy differs from treatment with streptomycin. In the group of children in Bellevue Hospital, no patient under treatment with isoniazid for any cause, even for miliary tuberculosis, has developed clinical meningitis even when tubercle bacilli have been cultured from the cerebrospinal fluid. Careful inquiry among other investigators has not revealed any instance of clinical meningitis developing while the patient was taking isoniazid.

If these observations are substantiated, it may radically change the present indications for specific therapy in primary tuberculosis. The use of isoniazid will have to be considered for every child with active primary tuberculosis and probably also for children with known recent conversion of tuberculin tests even if chest roentgenograms are normal. The duration of therapy would be for a year in order to cover the period during which meningitis would be most likely to develop.

Before entering into such an extensive program of antimicrobial therapy, it is important that a carefully controlled series of children with primary tuberculosis be studied, in which the patients to be treated are selected at random and all the children are followed for at least a year. At the present time such a series is being assembled in the chest clinic of the Children's Medical Service of Bellevue Hospital. Since the actual incidence of meningitis in primary tuberculosis is unknown, a very large number of cases may be required to demonstrate the effec-

tiveness of isoniazid in the prevention of meningitis. Many physicians are using chemotherapy in all children with primary asymptomatic tuberculosis, and others are treating a few cases without a fixed plan. A cooperative study using similar methods of selecting cases for antimicrobial therapy and a similar plan of treatment probably could easily be arranged under sponsorship such as that of the American Trudeau Society and might be most helpful in extending knowledge of the effectiveness of isoniazid in the prevention of serious complications of primary tuberculosis, especially meningitis.

The prognosis of primary tuberculosis will be greatly improved if meningitis can be prevented in known cases of primary tuberculosis. But it is important to realize that, although improvement in the specific therapy of tuberculosis may save lives and possibly prevent serious complications, we shall still be faced with the problem of the undiagnosed case which develops complications before infection is discovered.

Any program to improve the prognosis of primary tuberculosis by the wider use of antimicrobial therapy must be developed in coordination with plans for the prevention of tuberculous infection and for early diagnosis of established primary tuberculosis.

SUMMARY

Specific therapy has markedly reduced the mortality among children admitted to Bellevue Hospital with primary pulmonary tuberculosis. The mortality from 1930 through 1946 was 21.5 per cent. This fell to 5.0 per cent after the introduction of therapy with streptomycin combined with Promizole or para-aminosalicylic acid, even though only 35 per cent of the total number of patients received antimicrobial therapy. Since the introduction of isoniazid in 1952, the mortality rate has been further reduced to 1.5 per cent.

There is evidence in this reported series, as well as information from other workers, that isoniazid may prevent the development of complications of primary tuberculosis, especially meningitis. To investigate this important point, treated cases of primary pulmonary tuberculosis and untreated controls are being studied at Bellevue Hospital. This information can be obtained most rapidly by the cooperation of a larger group of investigators using similar protocols.

SUMARIO

El Efecto de la Terapéutica Antimicrobiana sobre el Pronóstico de la Tuberculosis Primaria en los Niños

La terapéutica específica ha rebajado notablemente la mortalidad entre los niños recibidos en el Hospital Bellevue con tuberculosis pulmonar primaria. La mortalidad desde 1930 hasta 1946 inclusive fué de 21.5 por ciento. Esto ha bajado a 5.0 por ciento desde la introducción de la terapéutica con estreptomicina combinada con Promizol o ácido para-aminosalicílico, aunque sólo 35 por ciento del total de enfermos recibieron terapéutica antimicrobiana. Desde la introducción de la isoniacida en 1952, la mortalidad ha sido reducida aun más a 1.5 por ciento.

Hay pruebas en esa serie comunicada, así como información procedente de otros técnicos, en el sentido de que la isoniacida puede impedir la aparición de complicaciones de la tuber-

culosis primaria, y especialmente meningitis. Para investigar este importante punto, se hallan en estudio en el Hospital Bellevue casos tratados de tuberculosis pulmonar primaria. Esta información puede obtenerse en la forma más rápida mediante la colaboración de un grupo mayor de investigadores que usen protocolos semejantes.

RESUME

L'effet de la thérapie antibactérienne sur le pronostic de la primo-infection tuberculeuse chez l'enfant

Le traitement spécifique a considérablement réduit la mortalité chez les enfants admis au "Bellevue Hospital" (New York) pour primo-infection tuberculeuse du poumon. De 1930 à 1946 inclusivement, le taux de la mortalité a été 21,5%. Il est descendu à 5% après introduction de la streptomycine conjuguée au Promizole ou au PAS, alors que 35% seulement de la totalité des malades recevaient la médication antibactérienne. Depuis l'introduction de l'isoniazide en 1952, le taux de mortalité a été encore réduit jusqu'à 1,5%.

Cette série d'observations ainsi que les observations recueillies par d'autres auteurs constituent la preuve que, dans la primo-infection tuberculeuse, l'isoniazide peut prévenir le développement de complications, en particulier de la méningite. Pour élucider ce point important, des recherches sont effectuées actuellement au "Bellevue Hospital" dans des cas de primo-tuberculose pulmonaire traités et dans des cas témoins, non traités. La coopération de groupes d'investigateurs plus nombreux utilisant des protocoles similaires pourrait donner cette information plus rapidement.

ARTIFICIAL CELLULAR IMMUNITY AGAINST TUBERCLE BACILLI[1]

An Effect of Polyoxyethylene Ethers (Triton)

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INTRODUCTION

Cornforth, Hart, Rees, and Stock (1) have shown that the non-ionic surface-acting substance, Triton WR-1339,[3] acts strongly against tuberculous infection in the mouse, and its antituberculous properties have been confirmed by Solotorovsky and Gregory (2). Cornforth and associates (1) allowed ethylene oxide to react with the polymeric condensation products of formaldehyde and *p-tert*-octylphenol to synthesize a homologous series of polyoxyethylene ethers of similar composition to Triton WR-1339. This mixture, designated D2, was less toxic than the commericial product, but of equal therapeutic activity.

Virulent strains of the tubercle bacillus will grow in liquid media containing D2 in concentrations as high as 1.0 per cent. In fact, Triton has been recommended as a substitute for Tween 80[4] in promoting dispersed growth of tubercle bacilli in liquid media (3, 4). On the other hand, avirulent variants of *M. tuberculosis*, for example, the strain H37Ra, are quite strongly inhibited by D2 when growing in liquid media. The effect of these substances on intraceullular tubercle bacilli *in vitro* has also been examined. In a study of the effects of antituberculous drugs on bacilli growing in monocytes in culture, the addition of 0.1 per cent of Triton WR-1339 to the monocyte culture medium had no effect on the growth of intracellular tubercle bacilli of a virulent strain (7).

Hart, Long, and Rees (6) found that therapeutically active polyoxyethylene ethers depress tuberculin sensitivity in tuberculous or vaccinated animals, but were not prepared to relate the desensitizing and antituberculous properties causally. Of the surface-active agents so far reported, all those with therapeutic activity also produce marked lipemia in treated animals. However, no correlation has been found between therapeutic activity and the degree of lipemia produced, and there is no evidence that the antituberculous activity of these substances depends on the disturbance of fat metabolism.

The polyoxyethylene ethers have insufficient bacteriostatic action against tubercle bacilli *in vitro* to account for their therapeutic effect; neither has serum from animals treated with these substances any bacteriostatic action *in vitro*. For this reason it has been suggested (8) that the drugs must, in some way, modify the host in its reponse to tubercle bacilli. In the experiments recorded

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[3] Triton is manufactured by the Rohm and Haas Co., Philadelphia, Pennsylvania.

[4] Tween 80 is the proprietary name for the polyoxyethylene derivative of Sorbitan monooleate, obtainable from the Atlas Powder Co., Wilmington, Delaware.

here, this problem was approached by comparing the behavior of tubercle bacilli in cultures of monocytes from control animals with that of bacilli in cultures of monocytes from animals treated with Triton. Monocytes and sera from rabbits treated with a therapeutically active sample of Triton WR-1339 were examined in the manner described in an earlier paper (9) for their capacity to support the growth of intracellular tubercle bacilli in monocyte cultures *in vitro*. In addition, the effect on bacterial growth, produced by the addition of Triton in various concentrations to the culture medium, of monocytes from normal untreated animals has been re-examined.

MATERIALS AND METHODS

Experiments were performed with both rabbit and guinea pig monocytes. Treated and control animals represented pairs taken from fully grown litter-mate stock of crossbred strains. The treated animals were given three injections of a 12.5 per cent solution of Triton WR-1339 at three-day intervals; each dose contained 300 mg. of Triton per kg. of body weight. The control animals received an equal volume of saline. Rabbits were injected intravenously, and guinea pigs subcutaneously, in the flank.

Mononuclear cell exudates, induced by the injection of liquid paraffin four days previously, were withdrawn from the peritoneal cavity of treated and control animals forty-eight hours after the last injection of Triton or saline.

The methods used for the preparation and maintenance of monocyte cultures infected with tubercle bacilli have been given in an earlier publication (7). The strains of tubercle bacilli used and the special procedures adopted here for quantitative parasitization of monocytes and for the measurement of bacterial growth in monocyte cultures have been set out in an earlier paper (9).

EXPERIMENTAL RESULTS

Growth of Tubercle Bacilli in Monocytes from Triton-Treated Animals

Three experiments were performed, two with monocytes from normal and Triton-treated rabbits and one multiple experiment with monocytes from 3 normal and 3 Triton-treated guinea pigs.

Peritoneal exudates were recovered from the Triton-treated animals at a time of intense lipemia, and the peritoneal washings were faintly opalescent after the removal of the cells. The monocytes from Triton-treated animals did not appear to differ morphologically from those of normal animals, either in the living state or in stained preparations. They did not appear to have ingested any of the lipoid material in their environment. It was observed, however, that fewer cells were present in the exudates of treated animals. The monocytes usually numbered only one-fifth or one-tenth of those present in the untreated control. In one attempted experiment, the exudate of a treated animal was almost cell free, although paraffin was present in the peritoneal cavity.

In the first experiment, two strains of tubercle bacilli, H37Rv and R1Rv, taken from sixteen-day-old Tween-albumin cultures, were used to infect monocytes from control and treated rabbits. Monocytes from each animal were infected with approximately equal numbers of bacilli of each strain. The culture medium contained 20 per cent normal serum in Hanks' solution without added Triton or antituberculous agent. Medium changes were made at intervals of twenty-four or forty-eight hours.

TABLE 1

NUMBERS OF BACILLI, STRAIN H37Rv, PRESENT IN CULTURES OF MONOCYTES FROM NORMAL AND TRITON-TREATED RABBITS AFTER ONE AND SIX DAYS' INCUBATION

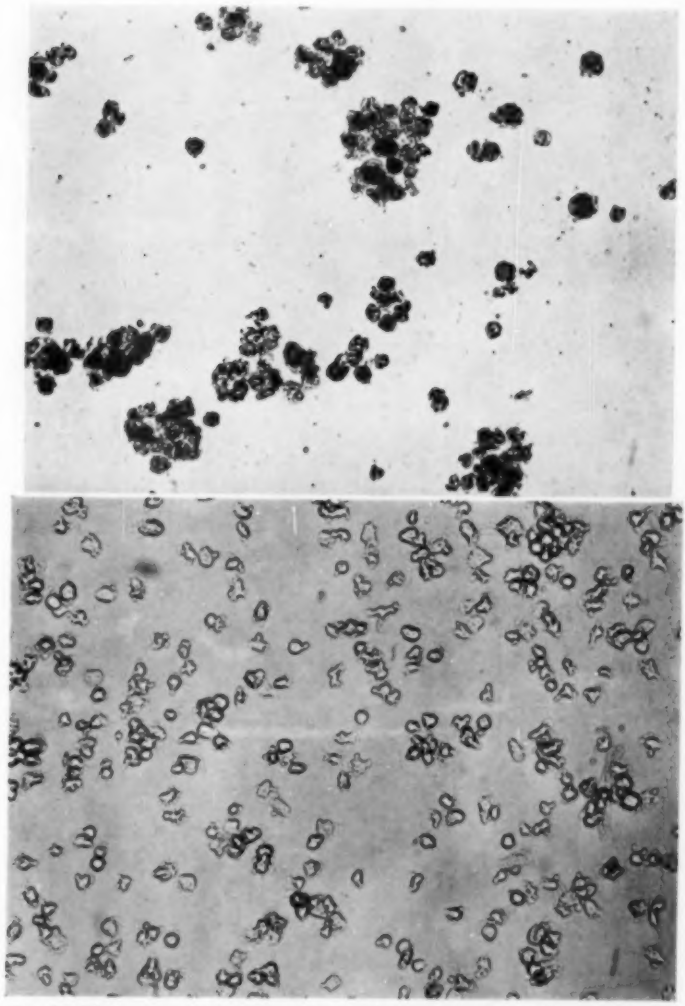
	PERIOD OF INCUBATION OF MONOCYTE CULTURE, DAYS			
	1		6	
	Source of Monocytes			
	Normal Rabbit	Triton-Treated Rabbit	Normal Rabbit	Triton-Treated Rabbit
Average number of bacilli per infected monocyte	3.3	3.5	20*	4.2
Average number of bacilli per monocyte of the total population	2.1	2.4	20	3.2
Percentage of monocytes infected	63	62	97	75
Percentage of monocytes containing more than 20 bacilli	0	0.3	92	7.7
Coefficient of increase			>9.5	1.3

* Death of a small number of monocytes had occurred prior to fixation.

Observations in cultures infected with the virulent strain H37Rv.: The data presented in table 1 show that slightly more bacilli were present in cultures of monocytes from the Triton-treated animal. Until the fifth day, all of the cultures remained in perfect condition. On the fifth day an occasional opaque cell was seen in the cultures of normal monocytes. On the sixth day such cells had become very numerous; they were absent from the monocytes of the Triton-treated animal. Cultures fixed on this day showed an apparent absence of growth in monocytes from the Triton-treated animal but a profuse growth of intracellular bacilli in monocytes from the control rabbit. In the normal culture the bacilli had increased at least tenfold, but growth had proceeded too far for an accurate estimate to be made. In the monocytes from the treated animal, on the other hand, there had been only a 30 per cent increase, and this was due entirely to growth of bacilli in a small proportion of the monocytes. In the majority no growth appeared to have taken place.

On the seventh day the difference between the two culture series was very conspicuous. In cultures of normal monocytes, almost all of the cells had died (figure 1), whereas monocytes in cultures from the Triton-treated animal remained in perfect condition (figure 2). The difference on the eighth day was even more pronounced (figures 3 and 4); growth of bacilli was now pronounced in cultures of normal monocytes (figure 5), but was still not evident in the monocytes from the treated rabbit (figure 6).

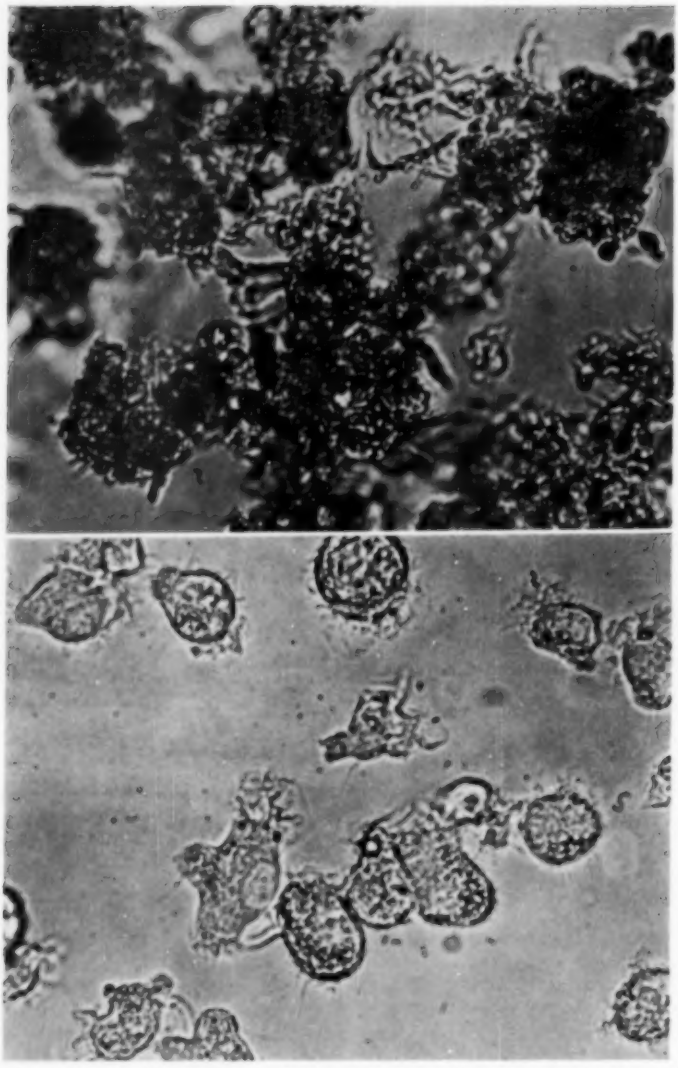
Inhibition of growth in monocytes from the Triton-treated animal was not absolute, and the first signs of degeneration were observed on the ninth day. Deterioration of the cultures, which was then fairly rapid, may have been hastened by dissemination through the culture of bacilli liberated from the small proportion of monocytes which had permitted growth of intracellular bacilli.



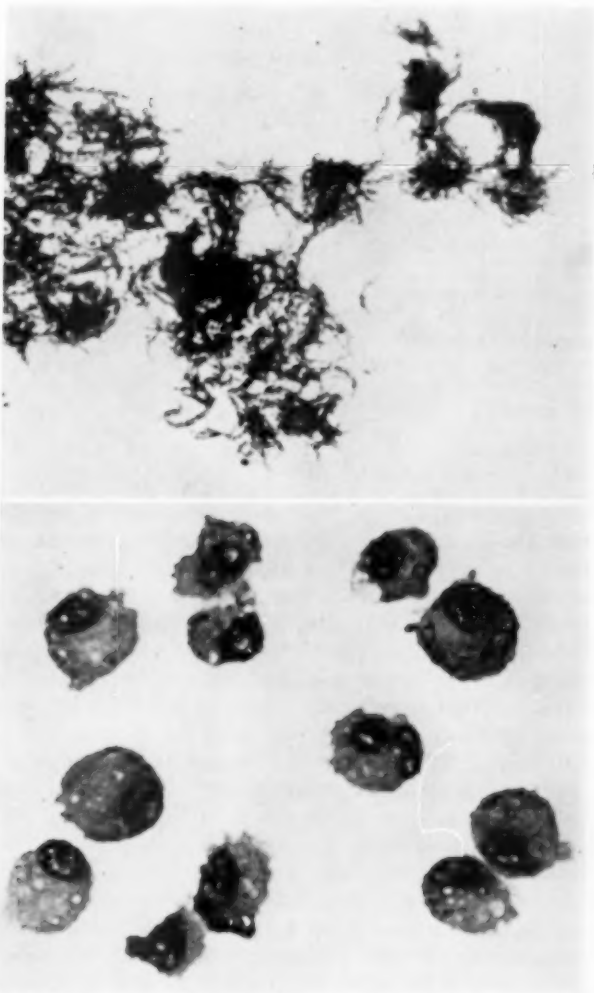
FIGS. 1 and 2. Living monocyte cultures infected with tubercle bacilli, strain H37Rv, showing the appearances after seven days' incubation in cultures of normal monocytes and monocytes from a Triton-treated rabbit. (Transmitted light, × 150).

FIG. 1 (Upper). Complete death of monocytes in cultures from the normal control rabbit.

FIG. 2 (Lower). Healthy motile monocytes in cultures from Triton-treated rabbit. The field contains one fibroblast (center right).



FIGS. 3 and 4. The same cultures as those seen in figures 1 and 2 after eight days' incubation. (Transmitted light, × 450).



FIGS. 5 and 6. Stained preparations of the cultures shown in figures 1 to 6; fixed on the eighth day of incubation. (Ziehl-Neelsen-methylene blue, × 680).

FIG. 5 (Upper). Proliferated mass of tubercle bacilli in culture of monocytes from the normal control.

FIG. 6 (Lower). Absence of bacterial multiplication in monocytes obtained from the Triton-treated rabbit.

TABLE 2

NUMBERS OF BACILLI, STRAIN R1Rv, PRESENT IN CULTURES OF MONOCYTES FROM NORMAL AND TRITON-TREATED RABBITS AFTER ONE, SIX, TEN, AND TWELVE DAYS' INCUBATION

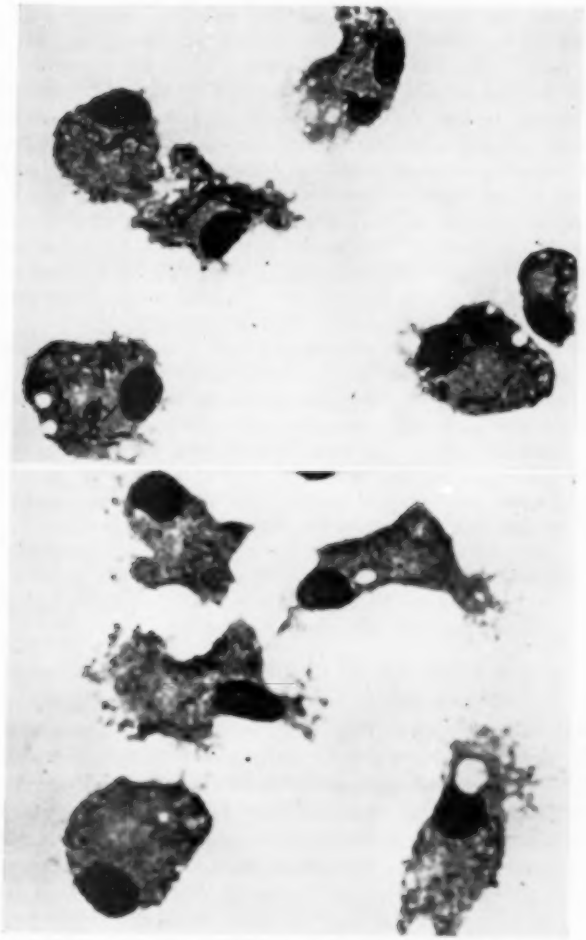
	PERIOD OF INCUBATION OF MONOCYTE CULTURE, DAYS							
	1		6		10		12	
	Source of Monocytes							
	C	T	C	T	C	T	C	T
Average number of bacilli per infected monocyte	3.8	4.2	4.4	2.5	8.9	2.7	12.6	3.8
Average number of bacilli per monocyte of the total population	2.2	2.5	2.6	1.1	6.1	1.2	10.6	1.6
Percentage of monocytes infected	59	60	59	45	69	44	84	42
Percentage of monocytes containing more than 20 bacilli	0.4	0	2.6	0	17	0	40	0.1
Coefficient of increase			1.2	0.5	2.8	0.5	4.8	0.6

C = control monocytes
T = monocytes from Triton-treated rabbit

Observations in cultures infected with attenuated bacilli of the strain R1Rv.: The data presented in table 2 show that initially more bacilli were present in the cultures of monocytes from the Triton-treated animal.

The living cultures were observed directly, and for a period of ten days no difference could be observed between cultures of normal monocytes and monocytes from the treated animal. On the tenth day, however, a small number, perhaps 10 per cent, of the normal monocytes had become opaque and granular—a change seen in monocytes containing large numbers of bacilli. By the twelfth day the difference between the two culture series was very marked. Almost half of the monocytes in cultures of normal cells had become granular, while the monocytes from the treated rabbit were entirely normal in appearance. The experiment was terminated on the twelfth day by transferring the remaining cultures from treated animals to oleic-acid–albumin agar to find out whether the bacilli remaining within the monocytes were still viable. Unfortunately, the cultures became desiccated, and no answer was obtained to this question.

The bacterial counts obtained in cultures after one, six, ten, and twelve days of incubation are recorded in table 2. They show that, while the bacterial population in normal monocytes had undergone a steady increase in numbers to almost fivefold on the twelfth day, the bacterial population in the monocytes from the Triton-treated animal had steadily declined. This is most convincingly shown by the fall in percentage of infected monocytes. The very slight increase in numbers which occurred between the tenth and twelfth days was due entirely to multiplication of bacilli in a monocyte here and there (about one in 1,000 of the monocyte population). This multiplication of bacilli in an occasional cell was not revealed in the count made on the tenth day, but inspection of the culture showed that two or three monocytes in the population of fifty or sixty thousand



FIGS. 7 and 8. Stained monocyte cultures fixed after ten days' incubation, showing the difference in the extent of bacterial multiplication of tubercle bacilli, strain R1Rv, in monocytes from the normal control (figure 7) and in monocytes from an animal treated with Triton (figure 8). In the latter, very few acid-fast bacilli were found; in their place there was a small amount of brown pigment. (Ziehl-Neelsen-methylene blue, × 680).

contained elongated proliferating bacilli. The remainder of the monocytes contained occasional acid-fast rods or cocci together with a variable deposit of a golden-brown pigment. Figures 7 and 8 are an attempt to show the difference in appearance on the tenth day of cultures of monocytes from normal and Triton-treated animals. The photomicrographs, however, do not show adequately the appearance of bacterial debris in the latter cells.

The second experiment on the growth of bacilli in monocytes from a Triton-treated animal was a composite experiment with monocytes from a normal control rabbit, a rabbit vaccinated with H37Rv, and a rabbit which had been treated with Triton for the previous nine days. Two strains of tubercle bacilli were used to infect the monocytes, virulent bovine-type Branch, and attenuated human-type R1Rv. Bacilli of these strains were prepared from three-week and seven-day Tween-albumin cultures, respectively. In anticipation of a retarded growth rate in monocytes from the treated animal, these were infected with an inoculum twice that of the control and "immune" monocytes. The culture chambers were filled with a suspension of monocytes denser than in the standard procedure (1.5 X), and the medium was changed every three days. Streptomycin (3.0 γ per ml.) was also incorporated in the culture medium. The serum from the Triton-treated animal in this experiment contained rather less lipoid than in the previous experiment.

In the same experiment an attempt was made to determine whether the lipemic serum of animals treated with Triton, when incorporated in the medium of monocyte cultures, could influence the growth of intracellular bacilli. It is generally recognized that lipemic serum is unsatisfactory for tissue culture purposes, and this is true for isolated monocyte cultures. To minimize toxicity, a sample of lipemic serum was stored for one month, during which time a layer of fat developed at the surface. The underlying serum was passed through a Seitz filter and incorporated in the usual nutrient medium at a concentration of 5 per cent. After two days' incubation the standard medium was changed in representative cultures from each series and replaced by medium containing added lipemic serum. Even at a concentration of 5 per cent and after much of the fat had been separated from it, the lipemic serum still imparted a dense opacity to the culture medium.

Observations in cultures infected with the virulent strain Branch: The data presented in table 3 show that at least twice as many bacilli had been incorporated in cultures of monocytes from the treated animal. The counts show that bacilli of the Branch strain, which normally grows more rapidly than any other strain so far tested in monocyte cultures, had grown less abundantly than in either the normal control or "immune" control cultures. In view of the heavy inoculum in monocytes from the treated rabbit, the difference appears to be significant. In addition, it was observed that the cultures of monocytes from the Triton-treated animal survived, as in the previous experiment with H37Rv, twice as long as the control cultures.

In each culture series, growth of intracellular bacilli was slightly less in the presence of lipemic serum, but it is doubtful whether the difference was significant.

Observations in cultures infected with the attentuated strain R1Rv.: The data presented in table 4 show that here, too, the number of bacilli present initially in monocytes from the Triton-treated animal was approximately twice that present in the cultures of normal and "immune" cells. The bacterial counts show that young bacilli of the strain R1Rv, which normally grow quite rapidly in an intracellular environment (9), had been almost totally inhibited for a period of eight days in the monocytes from the treated rabbit. At five days there was virtually no evidence of multiplication, and at eight days a small increase

TABLE 3

NUMBERS OF BACILLI, STRAIN BRANCH, PRESENT IN CULTURES OF MONOCYTES FROM NORMAL, VACCINATED, AND TRITON-TREATED RABBITS IN MEDIUM CONTAINING NORMAL SERUM AND MEDIUM TO WHICH LIPEMIC SERUM FROM A TRITON-TREATED ANIMAL HAS BEEN ADDED

Counts Made After Two, Four, and Six Days' Incubation

	SOURCE OF MONOCYTE											
	Normal Rabbit				Immunized Rabbit				Triton-Treated Rabbit			
	Medium											
	N/s			L/s	N/s			L/s	N/s			L/s
	Days of Incubation											
	2	4	6	6	2	4	6	6	2	4	6	6
Average number of bacilli per infected monocyte	3.7	5.6	9.9	7.9	3.6	5.1	9.2	6.7	6.0	6.8	9.7	8.3
Average number of bacilli per monocyte of the total population	2.5	4.4	8.4	6.4	2.3	4.1	7.8	5.2	5.1	6.1	9.2	7.4
Percentage of monocytes infected	66	79	85	80	64	79	84	77	86	91	94	90
Percentage of monocytes containing more than 20 bacilli	0	1.4	9.4	9.8	0	2.1	8.0	5.6	4.0	3.7	6.8	7.8
Coefficient of increase		1.8	3.4	2.6	—	1.8	3.4	2.3	—	1.2	1.8	1.4

N/s = medium containing normal serum only
L/s = medium to which lipemic serum was added

of 20 per cent was found. As in the previous experiment, this increase in bacterial numbers was due to multiplication of bacilli in a small proportion of the monocyte population. In this experiment exposure to the lipemic serum produced no effect at all on the rate of increase in bacterial numbers.

In a third experiment, an attempt was made to compare growth of intracellular bacilli in monocytes from normal and Triton-treated guinea pigs. Great difficulty has always been experienced in obtaining good survival of guinea pig monocytes *in vitro* by the method employed in these studies. The present experiment was no exception and was, therefore, only partially successful. Qualitatively, however, the results obtained confirmed the experiments with rabbit monocytes.

Only 2 animals in each group of 3 treated and 3 control guinea pigs yielded satisfactory exudates. Monocytes from each of the 4 guinea pigs were infected with approximately equal numbers of bacilli from a seven-day-old culture of strain R1Rv. Eight cultures were prepared from each series. All control cultures died on the fourth or fifth day of incubation. Cultures fixed and stained at these times showed massive growth of bacilli. Of the cultures from Triton-treated

TABLE 4

NUMBERS OF BACILLI, STRAIN R1Rv, PRESENT IN CULTURES OF MONOCYTES FROM NORMAL, VACCINATED, AND TRITON-TREATED RABBITS IN MEDIUM CONTAINING NORMAL SERUM AND MEDIUM TO WHICH LIPEMIC SERUM FROM A TRITON-TREATED ANIMAL HAD BEEN ADDED

Counts Made After One, Five, and Eight Days' Incubation

	SOURCE OF MONOCYTES											
	Normal Rabbit				Immunized Rabbit				Triton-Treated Rabbit			
	Medium											
	N/s			L/s	N/s			L/s	N/s			L/s
	Day of Incubation											
	1	5	8	8	1	5	8	8	1	5	8	8
Average number of bacilli per infected monocyte	6.3	7.5	13.6	13.3	4.6	6.3	11.6	11.5	7.7	7.9	8.5	Not available
Average number of bacilli per monocyte of the total population	4.3	6.4	12.6	12.1	3.0	4.8	10.7	10.2	6.4	7.1	7.7	
Percentage of monocytes infected	68	85	93	91	65	72	93	89	83	89	91	
Percentage of monocytes containing more than 20 bacilli	0.5	5.7	30	40	0.7	51	26	30	5.0	5.3	10.6	
Coefficient of increase		1.5	2.9	2.8	—	1.6	3.6	3.4	—	1.1	1.2	

N/s = medium containing normal serum only
L/s = medium to which lipemic serum was added

animals, the four which had not been fixed still contained numerous healthy motile monocytes on the ninth day of incubation; those which had been fixed showed no certain evidence of bacterial multiplication on the fifth or eighth day of incubation.

Since spontaneous death of monocytes had occurred continuously during incubation, it was not possible to measure, even approximately, the relative rates of increase. It was clear, however, that growth in monocytes from the Triton-treated animals had been greatly, and perhaps completely, inhibited.

The Effect of Triton on the Growth of Tubercle Bacilli in Monocytes from Untreated Animals

Several attempts were made to determine whether the addition of Triton to the culture medium would inhibit the growth of tubercle bacilli in monocytes from normal, untreated rabbits. In the experiments already cited (7), heavy inocula of a very rapidly growing strain of tubercle bacilli had been used, and Triton was tested at only one concentration.

In the present experiments, attempts were made to show a direct action of Triton on intracellular tubercle bacilli by varying the concentration of drug

TABLE 5

NUMBERS OF BACILLI PRESENT AFTER ONE, FIVE, AND NINE DAYS' INCUBATION IN CULTURES OF NORMAL MONOCYTES INFECTED WITH TUBERCLE BACILLI, STRAIN R1Rv, IN MEDIUM CONTAINING VARYING CONCENTRATIONS OF TRITON WR-1339

	PERIOD OF INCUBATION OF MONOCYTE CULTURE (DAYS)										
	1	5					9				
	Concentration of Triton (Volume Per 100 Ml.)										
	—	0.00	0.025	0.013	0.006	0.003	0.00	0.025	0.013	0.006	0.003
Average number of bacilli per infected monocyte	3.1	5.5	5.2	4.8	4.7	5.0	13.2	10.5	11.0	12.2	11.9
Average number of bacilli per monocyte of the total population	1.5	2.9	3.0	2.6	2.6	2.4	9.4	8.9	8.5	9.5	9.1
Percentage of monocytes infected	49	53	57	53	56	48	71	73	77	78	76
Percentage of monocytes containing more than 20 bacilli	0	2.4	1.4	0.4	2.2	1.7	29	22	19	21	20
Coefficient of increase	—	1.9	2.0	1.7	1.7	1.6	6.3	5.9	5.7	6.3	6.1

and by using smaller inocula of a more slowly growing strain of the tubercle bacillus.

Preliminary experiments gave the concentration of Triton which could be tolerated by isolated monocytes. At a concentration of 0.1 per cent, monocytes did not survive beyond the fifth day of incubation, and at 0.05 per cent, although surviving well, they became rounded and non-motile. At a concentration of 0.025 per cent, monocytes survived and behaved normally. In three further experiments, Triton had no direct inhibitory effect on the growth of intracellular tubercle bacilli; a representative experiment of this series is described below.

Monocytes from a normal animal were infected with a seven-day-old Tween-albumin culture of the attenuated strain R1Rv. Nutrient media were prepared containing Triton in final concentrations ranging from 0.025 to 0.003 per cent. Media were changed at twenty-four intervals for the first three days and at forty-eight-hour intervals thereafter. Fresh solutions of Triton were prepared for each medium change.

The number of bacilli present initially in the cultures, and after five and nine days of incubation, are shown in table 5. The counts show that at all concentrations of the drug the rates of increase in bacterial numbers were the same as those found in the untreated control cultures.

DISCUSSION

Certain surface-active polyoxyethylene ethers, although possessing little or no antituberculous activity *in vitro*, have a marked suppressive effect upon tuberculous infection *in vivo*. The present studies have revealed nothing con-

cerning the mechanism of this action, but they have shown a site at which the inhibitory action could take effect *in vivo*. Monocytes from animals which have been treated with a therapeutically active mixture of polyoxyethylene ethers appear altered in some way, so that tubercle bacilli which have been ingested by them are partially or totally inhibited in their growth, and may, in certain circumstances, be destroyed within the monocyte. In many experiments in which growth of virulent bacilli has been compared in similar conditions in monocytes from normal and vaccinated rabbits, or in monocytes from vaccinated rabbits which had or had not lost skin sensitivity to tuberculin, no instance has been found in which growth rates in the cells of the two animals have been significantly different. Moreover, bacilli of the strain R1Rv, which is more virulent than any other of the attenuated strains used in the present series of studies (9), have never before been seen to disappear in large numbers, as they did from monocytes of one of the Triton-treated rabbits.

The changes induced *in vivo* in the monocytes of animals treated with Triton endure for some time after the cells have been removed from the body. It is possible that the effects observed would be even more marked if a means could be found of keeping monocytes in isolation without exposing them to the serum of normal animals.

The mechanism of the action of Triton upon the host's mononuclear cells remains obscure. The toxicity of Triton imposes a strict limit to the concentration which can be sustained in the culture medium of isolated monocyte cultures. Within this limit it was impossible to demonstrate any direct effect on the growth of bacilli in monocytes from normal animals. A negative result from such an experiment is not conclusive, but it is doubtful whether more persuasive evidence could be obtained by the present method of study. It can only be said that it is unlikely that a surface-active agent of this type enters the cell and acts directly upon the intracellular organism. It seems justified, therefore, to adopt the tentative view that the monocyte becomes altered *in vivo* because of changes which occur in the body fluids of treated animals. The possibility remains that Triton enters the mononuclear cells under the conditions prevailing *in vivo*, but not under those prevailing in monocyte cultures *in vitro*.

The most obvious change in the host during treatment with a variety of surface-active agents is the disturbance which occurs in fat metabolism. The blood of treated animals becomes heavily charged with chylomicrons (10). These penetrate into the body fluids, and in these experiments were observed in the peritoneal washings obtained during the preparation of monocyte suspensions. Thus, the monocytes obtained from Triton-treated animals had existed in an environment containing large quantities of emulsified lipids. Had they ingested this dispersed fat they might be expected to have shown evidence of it microscopically, but they did not differ from normal monocytes in this respect.

When monocytes from either normal or Triton-treated animals were kept *in vitro* in medium containing lipemic serum, nothing appeared to be present in this serum which could affect the growth of intracellular bacilli. The lipemic

serum used had been freed from some of its fat content and had been Seitz filtered before use; moreover, it was present in the culture medium only in low concentrations mixed with normal serum. These precautions, necessary to avoid the cytotoxicity of lipemic serum, minimize the significance of the finding that bacilli grew as rapidly in cultures exposed to lipemic serum as in those exposed to normal serum.

SUMMARY

Surface-active polyoxyethylene ethers (Triton) have little action against tubercle bacilli *in vitro*, but act strongly against tuberculosis *in vivo*.

Tubercle bacilli grow slowly or not at all in monocytes derived from animals treated with Triton, although, in otherwise similar circumstances, the same bacilli grow freely in monocytes from untreated animals.

Treated animals develop intense lipemia, but the lipemic serum does not inhibit the growth of intracellular tubercle bacilli in the conditions of these experiments.

The action of Triton is thought to be indirect, for, when it is added to a culture of monocytes from untreated animals in the highest concentrations tolerated by these cells, it has no action against intracellular tubercle bacilli.

SUMARIO

Inmunidad Celular Artificial Contra los Bacilos Tuberculosos: Un Efecto de los Eteres Polioxietilénicos (Tritón)

Los éteres polioxietilénicos activos en la superficie (Tritón) ejercen poco efecto contra los bacilos tuberculosos *in vitro*, pero obran enérgicamente contra la tuberculosis *in vivo*.

Los bacilos tuberculosos proliferan en forma lenta o nula en monocitos derivados de animales tratados con Tritón, aunque, en circunstancias en lo demás semejantes, los mismos bacilos proliferan en grande en monocitos de animales no tratados.

Los animales tratados manifiestan intensa lipemia, pero el suero lipémico no inhibe el crecimiento de bacilos tuberculosos intracelulares en las condiciones de estos experimentos.

Se cree que la acción del Tritón es indirecta porque, cuando se agrega a un cultivo de monocitos procedentes de animales no tratados a las concentraciones más altas que toleran dichos glóbulos, no actúa contra los bacilos tuberculosos intracelulares.

RESUME

Immunité cellulaire artificielle envers le bacille tuberculeux: Un effet des éthers polyoxyéthylène (Triton)

Les éthers polyoxyéthylènes possédant une activité en surface (Triton) ont peu d'activité sur les bacilles tuberculeux *in vitro*, mais, *in vivo*, ils ont une action antituberculeuse marquée.

Les bacilles de Koch ne se développent que lentement ou pas du tout dans les monocytes prélevés chez les animaux traités par le Triton, cependant, dans des conditions par ailleurs semblables, ces mêmes bacilles croissent librement dans les monocytes de l'animal non traité.

Chez les animaux traités, on observe une lipémie intense, mais, dans les conditions de l'expérience, le sérum lipémique n'inhibe pas la croissance des bacilles tuberculeux intracellulaires.

On pense que l'action du Triton est indirecte car, lorsqu'il est ajouté à une culture de monocytes provenant d'animaux non traités et aux concentrations les plus fortes tolérées par ces cellules, il est sans action sur les bacilles tuberculeux intracellulaires.

Acknowledgments

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THE EFFECT OF BENZOATE AND SALICYLATE ON THE OXIDATIVE METABOLISM OF TUBERCLE BACILLI[1, 2]

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INTRODUCTION

Benzoic acid and some related compounds increase the metabolic activity of mycobacteria. Bernheim (1, 2, 3) has shown that the oxygen uptake of certain pathogenic strains is greatly accelerated when small amounts of benzoate or salicylate are added to bacterial suspensions in the resting state. These substances are not oxidized by pathogenic strains but are presumed to act as catalysts in some endogenous metabolic reaction of the cell. Oxygen uptake of avirulent tubercle bacilli and saprophytic strains was unaffected by salicylate, but benzoate was oxidized by the strains tested. Lehmann (4) confirmed the salicylate acceleration of pathogenic bacilli and postulated the use of this difference in oxygen consumption as a means of determining the pathogenicity of tubercle bacilli. It was these observations which led to the discovery of para-aminosalicylic acid.

Although the intermediate metabolism of benzoate and salicylate in the mycobacteria is obscure, their apparent importance in the metabolism of these organisms seemed to justify additional studies on a larger number of strains to determine whether a correlation does exist between the virulence of the organism and its stimulation by salicylate and benzoate.

MATERIALS AND METHODS

The following strains of virulent tubercle bacilli were tested: H37Rv, H37Rv-streptomycin-resistant, H37Rv-PAS-resistant, H37Rv-isoniazid-resistant, A-27, and Ravenel. Avirulent and attenuated strains included were H37Ra, R-1, BCG (ATCC 8420), BCG 317, BCG Paris, BCG Danish, BCG 913L, BCG 914K, BCG 873, BCG 805 (A-108), BCG 805 (B-108), and *Mycobacterium* 607.

For testing purposes, all strains were grown on the surface of Proskauer-Beck synthetic medium. The length of the growth period of the different cultures varied from four to fourteen days, depending upon the growth cycle of the individual strains, but in all instances only young vigorously growing cells in the logarithmic phase were employed. At the end of the growth period the cells were harvested and washed twice in normal physiologic saline. They were then suspended in a small amount of sterile 0.05 *M* phosphate buffer, pH 6.7, and transferred to a Ten Broeck tissue grinder in order to obtain a smooth suspension of cells. The organisms were washed once in the buffer solution and resuspended in an amount of buffer so that each milliliter of suspension contained 0.1 ml. of packed cells.

Oxygen uptake was measured in the Warburg apparatus, under air, at 37.5° C. in the usual manner (5). The vessels were allowed to equilibrate for fifteen minutes before 1 mg. of benzoate or salicylate was added from the side arm. Vessels were also run in which the cells had been preincubated for one hour with 20 γ of benzoate.

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[2] This investigation was supported by a research grant from the Committee on Medical Research of the American Trudeau Society, medical section of the National Tuberculosis Association.

RESULTS

The oxygen consumption of six of the BCG strains was accelerated by both salicylate and benzoate. The response in each case was immediate, and no further increase was shown by cells preincubated with 20 γ of benzoate. A "leveling off" of response by some strains in the presence of salicylate was not observed with

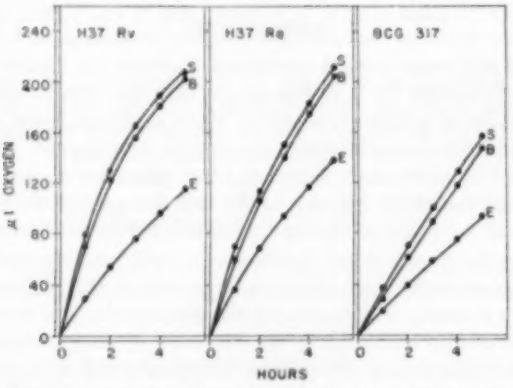

FIG. 1. The effect of 1.0 mg. of sodium salicylate and sodium benzoate on the oxygen consumption of virulent, avirulent, and attenuated tubercle bacilli.

TABLE 1

RESPONSE OF EIGHTEEN STRAINS OF TUBERCLE BACILLI TO 1.0 MG. OF SODIUM SALICYLATE AND SODIUM BENZOATE

STRAIN	VIRULENCE	BENZOATE	SALICYLATE
H37Rv	+	+	+
H37Rv-streptomycin-resistant	+	+	+
H37Rv-PAS-resistant	+	+	+
H37Rv-isoniazid-resistant	+	+	+
Ravenel	+	+	+
H37Ra	−	+	+
R-1	−	+	+
BCG 317	−	+	+
BCG Paris	−	+	+
BCG Danish	−	+	+
BCG 913 L	−	+	+
BCG 914 K	−	+	+
BCG 873	−	+	+
A-27	+	−	−
BCG 805 (A-108)	−	−	−
BCG 805 (B-108)	−	−	−
BCG ATCC 8420	−	⊕*	−
Mycobacterium 607	−	⊕*	−

* ⊕ = Oxidation of substrate

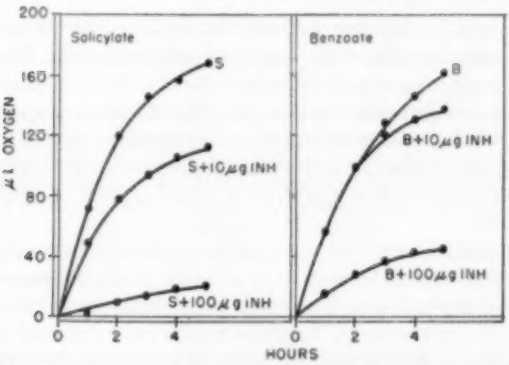

FIG. 2. The effect of isoniazid on the response of H37Rv tubercle bacilli to sodium salicylate and sodium benzoate. Endogenous values have been subtracted.

benzoate. Tendency for this "leveling off" to occur appeared to be greater in those strains with a high endogenous rate and was especially marked in the BCG Paris strain.

The H37Rv strain and its avirulent variant (H37Ra), the Ravenel bovine strain, and the R-1 also had an increased oxygen consumption in the presence of sodium benzoate and sodium salicylate (figure 1). Three strains, the virulent A-27, BCG 805 (A-108), and BCG 805 (B-108), showed no response to either benzoate or salicylate even though repeated attempts were made with very young cultures (table 1).

BCG 8420 from the American Type Culture Collection showed the typical response described by Bernheim. Oxygen consumption was unaffected by salicylate but showed a marked increase in the presence of benzoate. Unlike the other strains tested, there was a short initial lag period which could be eliminated by preincubation with a small amount of benzoate.

Isoniazid inhibits the response of H37Rv organisms to both benzoate and salicylate. This inhibition is demonstrable immediately, and there is no tendency for recovery. The endogenous respiration of the organism is inhibited only by the highest concentration of the drug tested (1,000 γ), whereas, in the presence of benzoate and salicylate, concentrations of 10 and 100 γ of isoniazid are also inhibitory. With 1,000 γ of isoniazid, increase in oxygen consumption in the presence of salicylate or benzoate is completely abolished (figure 2). Cells which had been rendered resistant to 10 γ of isoniazid were not affected by the drug even at the higher concentrations, but they continued to show an increased oxygen uptake in the presence of salicylate and benzoate.

DISCUSSION

The marked response that tubercle bacilli show to added benzoate or salicylate has long been one of the most interesting observations in studies of the oxidative metabolism of the mycobacteria. In the present study of a number of virulent, avirulent, and attenuated strains of tubercle bacilli of human and bovine origin,

no correlation could be detected between the virulence of the organism and its greater oxygen consumption with benzoate and salicylate. The H37Rv and H37Ra gave a similar response as did six of the nine BCG strains tested. Among the BCG strains showing this response was the 317 strain, reported by Yegian and Kurung (6) to be the most virulent on the basis of guinea pig inoculations and cord-formation studies. Also showing the response, however, were the Tice and Phipps strains (913 L, 914 K, and 873), which are low in virulence and show no cord formation.

As three of the strains showed no response to these substances, the salicylate and benzoate effect cannot be said to be a constant phenomenon of all tubercle bacilli. The age of the cells is an important factor in detecting this effect, yet even then other factors must enter in. Negative results were obtained with the highly virulent A27 strain as well as with the two 805 BCG cultures. The latter strains are said to be of slight virulence, showing poorly oriented cord pattern similar to that seen with R1Rv.

The only BCG strain tested which gave the response originally described by Bernheim was the ill-defined rapid-growing strain from the American Type Culture Collection which has been attenuated to the state of complete avirulence. It is impossible to say whether this loss of virulence was accompanied by metabolic changes which resulted in loss of ability to respond to salicylate accompanied by activation of an adaptive enzyme system capable of increasing oxygen consumption in the presence of benzoate and actually oxidizing this compound. Regardless of the mechanism involved, the findings emphasize again the importance of proper selection of strains for studies on the basic metabolism of tubercle bacilli and show that no distinction can be made between virulent and avirulent tubercle bacilli on the basis of the benzoate or salicylate effect.

Of interest is the finding that isoniazid depresses the organism's response to salicylate and benzoate. If, as stated by Bernheim, the increased oxygen consumption in the presence of these two aromatic acids is due to a catalysis of endogenous reactions, it is apparent that isoniazid inhibits these endogenous reactions. This observation is of especial interest when it is remembered that neither streptomycin nor *p*-aminosalicylic acid have any effect on either the endogenous respiration or the salicylate and benzoate response of virulent tubercle bacilli.

SUMMARY

A study has been made of eighteen strains of *Mycobacterium tuberculosis* to determine the effect of sodium salicylate and sodium benzoate on their oxidative metabolism. Organisms tested included virulent, avirulent, and attenuated organisms of human and bovine origin. Nine BCG organisms were tested.

The results obtained indicate that there is no correlation between the virulence of an organism and its oxidative response to salicylate and benzoate.

Isoniazid was found to inhibit the ability of the organism to respond to either salicylate or benzoate.

SUMARIO

El Efecto del Benzoato y del Salicilato sobre el Metabolismo Oxidante de los Bacilos Tuberculosos

Este estudio de dieciocho cepas del *Mycobacterium tuberculosis* tuvo por fin determinar el efecto del salicilato de sodio y del benzoato de sodio sobre el metabolismo oxidante de aquéllas. Los bacilos comprobados comprendían microbios virulentos, avirulentos y atenuados de procedencia humana y bovina. Hubo nueve BCG comprobados.

Los resultados obtenidos indican que no existe correlación entre la virulencia de un microbio y su respuesta oxidativa al salicilato y al benzoato.

Obsérvese que la isoniacida inhibía la capacidad del microbio para reaccionar ya al salicilato o al benzoato.

RESUME

Action du benzoate et du salicylate sur le métabolisme d'oxydation des bacilles tuberculeux

Il a été effectué une étude sur dix-huit souches de *Mycobacterium tuberculosis* pour déterminer l'action du salicylate de sodium et du benzoate de sodium sur leur métabolisme d'oxydation. Les bacilles testés comprenaient des germes virulents, avirulents et attenués d'origine humaine et bovine. Neuf organismes BCG ont été soumis aux tests.

Les résultats obtenus indiquent qu'il n'y a pas de corrélation entre la virulence d'un organisme et sa réaction d'oxydation au salicylate et au benzoate.

L'isoniazide a montré qu'elle inhibait la capacité de l'organisme à réagir à l'action soit du salicylate, soit du benzoate.

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TISSUE FATTY ACIDS AND THEIR POSSIBLE RELATIONSHIP TO THE NATURAL RESISTANCE OF RABBITS TO INFECTION WITH HUMAN-TYPE TUBERCLE BACILLI[1, 2, 3]

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INTRODUCTION

Rabbits occupy an unusual position among mammalian species in that, like cattle, they possess a rather high degree of resistance to tubercle bacilli of the human type and yet are extremely susceptible to the bovine type. Rich (1) and Lewis and Sanderson (2) inoculated rabbits intravenously with equal doses of human and bovine bacilli and killed the animals and examined the tissues with acid-fast stains at daily and weekly intervals thereafter. They were not able to detect any appreciable difference in the number of bacilli in the two series of animals during the first days, after which time the number of bovine bacilli began to surpass those of the human type and continued to do so progressively thereafter. Lurie and associates (3), by the use of colony counts made by seeding ground infected tissues on solid media, concluded that inhaled human-type tubercle bacilli multiply for only a short time in the rabbit lung and are then rapidly and completely destroyed. In natively susceptible rabbits the bacilli persist in large numbers for a much longer time.

It is tempting to postulate that the resistance of the rabbit is simply a manifestation of physiologic conditions in the normal animal which make it a more unfavorable environment for multiplication and/or survival of human-type bacilli. Investigations aimed at detecting humoral factors which might be responsible for the resistance of the rabbit to human-type tubercle bacilli have been concerned, for the most part, with attempts to demonstrate the ability of serum to inhibit multiplication of the organisms *in vitro*. The results of such studies have been variable (4–8).

It appeared possible that, through the use of *in vitro* growth studies more exhaustive than those reported previously, more exact information could be obtained whether in the normal rabbit there is some peculiarity of composition of tissues which presents a more unfavorable environment for multiplication and/or survival of human-type bacilli.

[1] From the Tuberculosis Research Laboratory, Veterans Administration Hospital, Washington, D. C.

[2] These studies were conducted in the laboratories of the Tuberculosis Unit, Communicable Disease Center, Chamblee, Georgia.

[3] This paper represents a portion of a dissertation submitted to the Graduate School of the University of Minnesota in partial fulfillment of requirements for the degree of Doctor of Philosophy.

EXPERIMENTAL

Strains of tubercle bacilli: Standard strains[4] of human (H37Rv) and bovine (Ravenel) tubercle bacilli were used throughout the course of the studies. The test strains were subcultured at weekly intervals in 250 ml. Erlenmeyer flasks containing 100 ml. of a modified Proskauer and Beck liquid medium (9).

Typical virulence of the test organisms was confirmed by animal inoculation every three months. A portion of the pellicle from a seven- to ten-day-old culture on the modified Proskauer and Beck medium was ground and standardized to contain 0.1 mg. per ml. according to the technique described by Youmans and Karlson (9). Albino rabbits weighing 2,000 to 3,000 gm. and mixed-color guinea pigs weighing 250 to 400 gm. were employed for determinations of virulence. The animals were ordinarily inoculated in pairs with 0.1 mg. of either the H37Rv or Ravenel strain of tubercle bacilli. Guinea pigs were inoculated subcutaneously in the right groin; rabbits, intravenously in the marginal vein of the ear.

Studies on Extracts of Normal Rabbit Lung Tissue

Four normal rabbits were killed by occipital trauma. The lungs were removed immediately, washed free of superficial blood with distilled water, and frozen in a carbon dioxide box at −70°C. On the following day the specimens were thawed, pooled, and weighed (41 gm.). For initial fractionation of the tissue, a technique was used which was similar to that described by Awapara (10) for the estimation of free amino acids in tissues of the rat. The pooled rabbit lungs were homogenized in a Waring Blendor with five volumes of hot distilled water. Approximately three volumes of 95 per cent ethyl alcohol were added, and the precipitated protein was removed by filtration through Whatman No. 1 filter paper on a Buchner funnel. The filtrate was then mixed with three times its volume of chloroform, shaken vigorously, and centrifuged at 2,000 r.p.m. for fifteen minutes. The clear, top aqueous layer was removed, filtered through Whatman No. 1 filter paper, and evaporated to dryness at 50°C. A solid residue weighing 624 mg. was thus obtained. This material was then redissolved in distilled water in a final concentration of 50 mg. per ml., sterilized by passing it through a Coors (P3) filter, and stored at 5°C.

The alcohol-chloroform layer was filtered through two layers of Whatman No. 1 filter paper and evaporated to dryness in a fume hood with the aid of gentle heat. The dried residue weighed 983 mg. This material was then taken up in 15 ml. of warm 95 per cent ethyl alcohol which was subsequently evaporated to a volume of approximately 2 ml. To this were added approximately 50 ml. of acetone, and the precipitated phospholipids were separated by centrifugation at 3,000 r.p.m. for fifteen minutes. After removal of the supernatant acetone layer, the phospholipids were redissolved in warm alcohol and reprecipitated with fresh acetone. The pooled acetone washings and the precipitated phospholipids were both taken to dryness and found to weigh 418 mg. and 474 mg., respectively. Each of these fractions was redissolved in a 1:1 mixture of 95 per cent ethyl alcohol and chloroform to give a final concentration of 50 mg. per ml. and stored at 5°C.

For growth studies, eight-day-old cultures of the H37Rv and Ravenel strains which had been grown on the Proskauer and Beck liquid medium were harvested, ground, and standardized to contain 2 mg. per ml. Twofold serial dilutions of the three tissue extracts were then prepared, using as a diluent either sterile distilled water for the aqueous extract or a 1:1 mixture of 95 per cent ethyl alcohol and chloroform for the phospholipids and non-phospholipids. The various dilutions were added in 0.25 ml. amounts to a series of tubes containing 5 ml. of Proskauer and Beck medium. Control tubes received 0.25 ml. amounts of diluent alone. Since the phospholipid and non-phospholipid extracts had not been sterilized previously, the tubes of medium containing these materials were autoclaved for eight

[4] Obtained from Mr. William Steenken, Jr., Standard Culture Depot, Trudeau, New York.

minutes at 15 pounds of pressure (121°C.). The tubes were then inoculated with 0.1 ml. (0.2 mg.) of either the H37Rv or Ravenel suspension. Growth was read visually after seven, fourteen, twenty-one, and twenty-eight days' incubation at 37°C. and recorded by means of an arbitrary scale from 1-plus to 4-plus.

From the results presented in tables 1, 2, and 3, it may be seen that, whereas the aqueous extract and the phospholipid fraction supported growth of both the human and bovine tubercle bacilli even in relatively high concentrations, the nonphospholipid (acetone soluble) material was markedly inhibitory, but more so for the human than for the bovine organisms (table 3). Theoretically, the

TABLE 1

GROWTH RESPONSE OF HUMAN (H37Rv) AND BOVINE (RAVENEL) TUBERCLE BACILLI IN THE PRESENCE OF AN AQUEOUS EXTRACT OF NORMAL RABBIT LUNG

STRAIN	FINAL CONCENTRATION OF EXTRACT (mg. per ml.)	AMOUNT OF GROWTH—DAYS OF INCUBATION			
		7	14	21	28
H37Rv	2.6	—	—	—	—
	1.3	—	+	+	++
	0.65	+	+++	++++	++++
	0.325	+	+++	++++	++++
	Control	+	+++	++++	++++
	Control	+	+++	++++	++++
Ravenel	2.6	—	—	—	—
	1.3	+	+	+	+
	0.65	+	++	++	++
	0.325	+	++	+++	++++
	Control	+	+++	++++	++++
	Control	+	+++	++++	++++

TABLE 2

GROWTH RESPONSE OF HUMAN (H37Rv) AND BOVINE (RAVENEL) TUBERCLE BACILLI IN THE PRESENCE OF THE PHOSPHOLIPIDS OF NORMAL RABBIT LUNG

STRAIN	FINAL CONCENTRATION OF EXTRACT (mg. per ml.)	AMOUNT OF GROWTH—DAYS OF INCUBATION			
		7	14	21	28
H37Rv	2.6	+	+++	++++	++++
	1.3	+	+++	++++	++++
	0.65	+	+++	++++	++++
	0.325	+	+++	++++	++++
	Control	+	+++	++++	++++
	Control	+	+++	++++	++++
Ravenel	2.6	+	+++	++++	++++
	1.3	+	+++	++++	++++
	0.65	+	+++	++++	++++
	0.325	+	+++	++++	++++
	Control	+	+++	++++	++++
	Control	+	+++	++++	++++

TABLE 3

GROWTH RESPONSE OF HUMAN (H37Rv) AND BOVINE (RAVENEL) TUBERCLE BACILLI IN THE PRESENCE OF THE NON-PHOSPHOLIPIDS OF NORMAL RABBIT LUNG

STRAIN	FINAL CONCENTRATION OF EXTRACT (mg. per ml.)	AMOUNT OF GROWTH—DAYS OF INCUBATION			
		7	14	21	28
H37Rv	0.16	—	—	—	—
	0.08	—	—	—	—
	0.04	—	—	—	—
	0.02	—	—	—	—
	0.01	—	—	—	—
	0.005	—	—	±	+
	Control	±	+	+++	+++
	Control	±	+	+++	+++
Ravenel	0.16	—	—	—	—
	0.08	—	—	—	—
	0.04	—	—	—	—
	0.02	—	—	±	+
	0.01	—	—	+	++
	0.005	±	++	+++	+++
	Control	±	++	+++	+++
	Control	±	++	+++	+++

acetone-soluble material should consist primarily of neutral fat, sterols, and traces of free fatty acids. In view of what is known concerning the toxicity for tubercle bacilli of even minute traces (0.1 to 1γ per ml. of medium) of certain naturally occurring fatty acids (11), it was decided to conduct growth studies similar to those just described but confined to an investigation of the effect of total fatty acids extracted from rabbit lung tissue.

Phospholipid and non-phospholipid fatty acids were isolated from the pooled lungs of three normal rabbits according to a technique similar to that described by Goldman and associates (12). For measuring the *in vitro* growth response of human- and bovine-type tubercle bacilli in the presence of these fatty acid preparations, ten-day-old cultures of the H37Rv and Ravenel strains grown on Proskauer and Beck medium were harvested, ground, and standardized to contain 5 mg. per ml. Twofold serial dilutions of the two fatty acid preparations were made, using ethyl ether as a diluent. The various dilutions were then added in 0.25 ml. amounts to a series of tubes containing 5 ml. of Proskauer and Beck medium. Control tubes received 0.25 ml. of ethyl ether, and all tubes were then autoclaved for eight minutes at 15 pounds of pressure. The tubes were inoculated with 0.1 ml. (0.5 mg.) of either the H37Rv or Ravenel suspension, incubated at 37°C., and the results were read and recorded in the usual fashion.

The results presented in tables 4 and 5 indicate that the fatty acids derived from both the phospholipids and nonphospholipids of normal rabbit lung are bacteriostatic for both strains of tubercle bacilli in extremely minute quantities. Moreover, there again was a difference in response demonstrating a greater susceptibility of the human-type organisms.

It appeared that it might be of some value to determine whether the activity of the mixture of fatty acids from normal rabbit lung might be attributable

TABLE 4

GROWTH RESPONSE OF HUMAN (H37Rv) AND BOVINE (RAVENEL) TUBERCLE BACILLI IN THE PRESENCE OF THE NON-PHOSPHOLIPID FATTY ACIDS FROM NORMAL RABBIT LUNG

STRAIN	FINAL CONCENTRATION OF FATTY ACID (γ per ml.)	AMOUNT OF GROWTH—DAYS OF INCUBATION			
		7	14	21	28
H37Rv	7.5	—	—	—	—
	3.7	—	—	—	—
	1.9	—	—	—	—
	0.9	—	++	+++	++++
	0.4	—	+++	++++	++++
	0.2	++	++++	++++	++++
	0.1	++	++++	++++	++++
	Control	++	++++	++++	++++
	Control	++	++++	++++	++++
Ravenel	7.5	—	—	—	—
	3.7	—	—	—	—
	1.9	+++	++++	++++	++++
	0.9	+++	++++	++++	++++
	0.4	+++	++++	++++	++++
	0.2	+++	++++	++++	++++
	0.1	+++	++++	++++	++++
	Control	+++	++++	++++	++++
	Control	+++	++++	++++	++++

TABLE 5

GROWTH RESPONSE OF HUMAN (H37Rv) AND BOVINE (RAVENEL) TUBERCLE BACILLI IN THE PRESENCE OF THE PHOSPHOLIPID FATTY ACIDS FROM NORMAL RABBIT LUNG

STRAIN	FINAL CONCENTRATION OF FATTY ACID (γ per ml.)	AMOUNT OF GROWTH—DAYS OF INCUBATION			
		7	14	21	28
H37Rv	7.5	—	—	—	—
	3.7	—	—	—	—
	1.9	—	—	—	—
	0.9	—	—	—	—
	0.4	++	+++	++++	++++
	0.2	++	++++	++++	++++
	0.1	++	++++	++++	++++
	Control	++	++++	++++	++++
	Control	++	++++	++++	++++
Ravenel	7.5	—	—	—	—
	3.7	—	—	—	—
	1.9	++	++++	++++	++++
	0.9	++	++++	++++	++++
	0.4	++	++++	++++	++++
	0.2	++	++++	++++	++++
	0.1	++	++++	++++	++++
	Control	++	++++	++++	++++
	Control	++	++++	++++	++++

solely to either its steam volatile or nonvolatile components. As the phospholipids, because of their relative preponderance in the lung tissue, afforded a more ample supply of material with which to work, they were used exclusively in the following investigations.

Steam volatile and nonvolatile fatty acids were prepared from a fresh sample of lung phospholipid by a method similar to that described by Lewkowitsch (13). The growth response of the human- and bovine-type tubercle bacilli in the presence of twofold serial dilutions of these acids was measured by the technique described previously.

It will be seen from the results presented in tables 6 and 7 that both the volatile and nonvolatile fatty acids isolated from the phospholipid of normal rabbit lung are bacteriostatic for the human- and bovine-type tubercle bacilli *in vitro*. The findings would also seem to indicate that slightly greater concentrations of both groups of fatty acids are required to inhibit completely the growth of the Ravenel strain than are needed to inhibit completely growth of the H37Rv strain.

In an attempt to characterize further the active component (or components) of the mixed fatty acids from the lung phospholipid, it was decided to investigate the activity *in vitro* of the saturated as compared with the unsaturated fatty acids in the mixture. For this purpose, modifications in technique were introduced which included a more vigorous saponification. It was thought that this might assure a more adequate recovery of fatty acids. The methods used were essentially those of Volk, Millington, and Weinhous (14). The lead salt procedure used for the separation of saturated from unsaturated acids was similar to that

TABLE 6

GROWTH RESPONSE OF HUMAN (H37Rv) AND BOVINE (RAVENEL) TUBERCLE BACILLI IN THE PRESENCE OF THE VOLATILE FATTY ACIDS FROM NORMAL RABBIT LUNG

STRAIN	FATTY ACID FINAL DILUTION	AMOUNT OF GROWTH—DAYS OF INCUBATION			
		7	14	21	28
H37Rv	1:10	—	—	—	—
	1:20	—	—	—	—
	1:40	—	—	—	—
	1:80	++	+++	++++	++++
	1:160	++	+++	++++	++++
	1:320	++	+++	++++	++++
	Control	++	+++	++++	++++
	Control	++	+++	++++	++++
Ravenel	1:10	—	—	—	—
	1:20	—	—	—	—
	1:40	±	+	+	+
	1:80	++	+++	++++	++++
	1:160	++	+++	++++	++++
	1:320	++	+++	++++	++++
	Control	++	+++	++++	++++
	Control	++	+++	++++	++++

TABLE 7

GROWTH RESPONSE OF HUMAN (H37Rv) AND BOVINE (RAVENEL) TUBERCLE BACILLI IN THE PRESENCE OF THE NON-VOLATILE FATTY ACIDS FROM NORMAL RABBIT LUNG

STRAIN	FATTY ACID FINAL DILUTION	AMOUNT OF GROWTH—DAYS OF INCUBATION			
		7	14	21	28
H37Rv	1:640	—	—	—	—
	1:1280	—	—	—	—
	1:2560	—	—	—	—
	1:5120	—	—	—	—
	1:10,240	—	—	—	—
	1:20,480	++	+++	++++	++++
	Control	++	+++	++++	++++
	Control	++	+++	++++	++++
Ravenel	1:640	—	—	—	—
	1:1280	±	±	±	+
	1:2560	±	±	±	+
	1:5120	±	±	±	+
	1:10,240	++	+++	++++	++++
	1:20,480	++	+++	++++	++++
	Control	++	+++	++++	++++
	Control	++	+++	++++	++++

TABLE 8

GROWTH RESPONSE OF HUMAN (H37Rv) AND BOVINE (RAVENEL) TUBERCLE BACILLI IN THE PRESENCE OF THE SATURATED FATTY ACIDS FROM THE PHOSPHOLIPIDS OF NORMAL RABBIT LUNG

STRAIN	FATTY ACID FINAL DILUTION	AMOUNT OF GROWTH—DAYS OF INCUBATION		
		7	14	21
H37Rv	1:20	—	—	—
	1:40	±	±	+
	1:80	±	±	+
	1:160	++	+++	++++
	1:320	++	++++	++++
	1:640	++	++++	++++
	1:1280	++	++++	++++
	Control	++	++++	++++
	Control	++	++++	++++
Ravenel	1:20	±	+	++
	1:40	+	+	++
	1:80	++	++	+++
	1:160	++	+++	++++
	1:320	++	++++	++++
	1:640	++	++++	++++
	1:1280	++	++++	++++
	Control	++	++++	++++
	Control	++	++++	++++

TABLE 9

GROWTH RESPONSE OF HUMAN (H37Rv) AND BOVINE (RAVENEL) TUBERCLE BACILLI IN THE PRESENCE OF THE UNSATURATED FATTY ACIDS FROM THE PHOSPHOLIPIDS OF NORMAL RABBIT LUNG

STRAIN	FATTY ACID FINAL DILUTION	AMOUNT OF GROWTH—DAYS OF INCUBATION		
		7	14	21
H37Rv	1:20	—	—	—
	1:40	—	—	—
	1:80	—	—	—
	1:160	—	—	—
	1:320	—	—	—
	1:640	—	+	+
	1:1280	++	++++	++++
	Control	++	++++	++++
	Control	++	++++	++++
Ravenel	1:20	—	—	—
	1:40	—	—	—
	1:80	—	—	—
	1:160	±	+	++
	1:320	+	+++	+++
	1:640	++	++++	++++
	1:1280	++	++++	++++
	Control	++	++++	++++
	Control	++	++++	++++

described by Hilditch (15). *In vitro* studies of the growth response of the two types of tubercle bacilli in the presence of the saturated and unsaturated fatty acids were performed in the usual fashion. From the results presented in tables 8 and 9, it will be seen that again the bovine tubercle bacilli appeared to be less susceptible than the human type to the *in vitro* bacteriostatic action of the fatty acid fractions, whether saturated or unsaturated.

Enzyme Studies

Any assumption that the fatty acid components of the rabbit lung might be determinants of the outcome of infection with human- and bovine-type tubercle bacilli implies a necessity that enzymes capable of removing fatty acids from the phospholipid molecule be present. As possible sources for these enzymes, it seemed logical to investigate both the infecting organisms and the lung tissue itself.

Phospholipase of tubercle bacilli: Both culture filtrates and homogeneous suspensions of the H37Rv and Ravenel strains of tubercle bacilli were examined for phospholipase activity. For the study of culture filtrates, a technique was chosen which was modified from that used by Kendall and associates (16) to demonstrate a soluble lipase in broth cultures of tubercle bacilli. For use as a substrate, a 3 per cent suspension of lung phospholipid was prepared in phosphate buffer at pH 7.0.

TABLE 10

RESULTS OF EXPERIMENTS DESIGNED TO DEMONSTRATE PHOSPHOLIPASE ACTIVITY IN CULTURE FILTRATES OF HUMAN (H37Rv) AND BOVINE (RAVENEL) TUBERCLE BACILLI

TUBE	CULTURE FILTRATE	SUBSTRATE	PHOSPHATE BUFFER (pH 7.0)	PROSKAUER AND BECK MEDIUM	ML. OF N/50 SODIUM HYDROXIDE*
Controls	1 ml. (H37Rv)		2 ml.		0.062
	1 ml. (Ravenel)		2 ml.		0.059
		2 ml.		1 ml.	0.370
		2 ml.	1 ml.		0.210
Experimental	1 ml. (H37Rv)	2 ml.			0.230
	1 ml. (H37Rv)	2 ml.			0.132
	1 ml. (Ravenel)	2 ml.			0.202
	1 ml. (Ravenel)	2 ml.			0.175

* The tubes were shaken continuously in a Fisher Kline-Test Rotator for forty-eight hours at 37°C., removed from the incubator, and their contents titrated to the neutral point with N/50 sodium hydroxide.

Experimental and control tubes, each in a total volume of 3 ml., were set up as shown in table 10. To each was added 0.5 ml. of toluene for the purpose of eliminating the possibility of bacterial contamination. The tubes were shaken continuously in a Fisher Kline-Test Rotator for forty-eight hours at 37°C., removed from the incubator, and their contents titrated to the neutral point with N/50 sodium hydroxide, using phenolphthalein as an indicator.

An examination of the last column in table 10 indicates no detectable phospholipase activity on the part of culture filtrates prepared from either the H37Rv or Ravenel strain. The acidity which developed in the experimental tubes was no greater than that demonstrated in the controls.

Homogeneous suspensions of the experimental organisms were examined for phospholipase activity by a method which was similar, except for certain refinements, to that used by Toda (17) in studies on the lecithinase of various bacteria. A 3 per cent suspension of the lung phospholipid in phosphate buffer at pH 7.0 was sterilized by heating to 70°C. on three successive days. This sterile material served as a substrate in the enzyme studies to be described. Sterile phosphate buffer (pH 7.0) was prepared by filtration through a sintered glass (UF) filter. Fifteen-day-old cultures of the H37Rv and Ravenel strains of tubercle bacilli growing in the Proskauer and Beck medium were harvested, ground, and standardized in the usual fashion to contain 3 mg. per ml., using the sterile buffer as a diluent.

Experimental and control tubes were prepared aseptically according to the scheme shown in table 11. The tubes were shaken continuously in the rotator for forty-eight hours at 37°C., removed from the incubator, and heated to 70°C. for one hour in order to kill the organisms. The fatty acids were extracted from the contents of each tube by the technique of Fairbairn (18) and were taken up in 10 ml. of 95 per cent redistilled ethyl alcohol. The solution was heated to the boiling point and titrated with N/50 sodium hydroxide, using phenolphthalein as an indicator. The titration value for 10 ml. of 95 per cent ethyl alcohol was obtained and subtracted from readings obtained with each of the experimental and control tubes.

TABLE 11

RESULTS OF EXPERIMENT DESIGNED TO DEMONSTRATE PHOSPHOLIPASE ACTIVITY IN HOMOGENEOUS SUSPENSIONS OF HUMAN (H37Rv) AND BOVINE (RAVENEL) TUBERCLE BACILLI

TUBE	H37Rv SUSPENSION	RAVENEL SUSPENSION	STERILE SUBSTRATE	STERILE PHOSPHATE BUFFER (pH 7.0)	ML. OF N/50 SODIUM HYDROXIDE*
Controls			2 ml.	1 ml.	0.292
			2 ml.	1 ml.	0.260
	1 ml.			2 ml.	0.035
		1 ml.		2 ml.	0.032
Experimental	1 ml.		2 ml.		0.305
	0.5 ml.		2 ml.	0.5 ml.	0.275
		1 ml.	2 ml.		0.370
		0.5 ml.	2 ml.	0.5 ml.	0.260

* The tubes were shaken continuously in the rotator for forty-eight hours at 37°C., removed from the incubator, and heated to 70°C. for one hour. Fatty acids were extracted from the contents of each tube and were titrated to the neutral point with N/50 sodium hydroxide.

TABLE 12

RESULTS OF EXPERIMENT DESIGNED TO DEMONSTRATE PHOSPHOLIPASE ACTIVITY IN NORMAL RABBIT LUNG TISSUE

TUBE	ENZYME PREPARATION	SUBSTRATE	PHOSPHATE BUFFER (pH 7.0)	ML. OF N/50 SODIUM HYDROXIDE*
Control	1 ml.		3 ml.	0.080
		2 ml.	2 ml.	0.635
Experimental	1 ml.	2 ml.	1 ml.	1.228
	1 ml.	2 ml.	1 ml.	1.318
	2 ml.	2 ml.		1.320

* The tubes were shaken continuously in the rotator for forty-eight hours at 37°C. and removed from the incubator. Fatty acids were extracted from the contents of each tube and were titrated to the neutral point with N/50 sodium hydroxide.

The results presented in table 11 show no appreciable acidity which could be ascribed to phospholipase activity on the part of either the H37Rv or Ravenel organisms.

Phospholipase of rabbit lung tissue: Several methods were used, with varying degrees of success, to prepare phospholipase from normal rabbit lung. The one found to be most satisfactory was that of Belfanti and Arnaudi as described by Francioli and Ercoli (19). As a substrate, a 3 per cent suspension of lung phospholipid in phosphate buffer (pH 7.0) was used.

Experimental and control tubes, each in a total volume of 4 ml., were set up as indicated in table 12. To each was added 0.5 ml. of toluene. The tubes were shaken for forty-eight hours at 37° C. and removed from the incubator. The

fatty acids were extracted as usual and titrated with N/50 sodium hydroxide, using phenolphthalein as an indicator. The results are shown in table 12, where it may be seen that normal rabbit lung tissue is possessed of readily detectable phospholipase activity.

DISCUSSION

The demonstration that fatty acids extracted from normal rabbit lung are extremely toxic for tubercle bacilli was not particularly surprising. The work of Dubos and Davis (11) has been mentioned previously. Much earlier, McJunkin (20) had reported that oleic acid manifests a profound effect on cultures of human-type tubercle bacilli which have been dehydrated with alcohol. Formation of granules, loss of acid-fastness, and complete solution of many of the bacilli occurred. Boissevain (21), in 1926, confirmed these findings but found that the preliminary dehydration was unnecessary. Tubercle bacilli lost their acid-fastness, and were apparently killed, by treatment *in vitro* with certain fatty acids of the aliphatic series. The effect seemed to depend on the presence of a long carbon chain (5 carbons) and a —COOH group and to increase with the number of double bonds. Linolenic acid was the most effective *in vitro*. Platonov (22, 23) noted that fats of the unsaturated type act bacteriolytically, retard the growth of tubercle bacilli, and lower their virulence. Guinea pigs inoculated with tubercle bacilli, the virulence of which had been lowered by exposure *in vitro* to unsaturated fats, developed a chronic tuberculosis of a fibrous character.

Toda (17), whose work has been mentioned earlier, was of the opinion that fatty acids split off from lecithin through the action of lecithinase or through natural decomposition are capable of destroying the acid-fastness of tubercle bacilli. Further, it has been reported (24) that, after saponification of phospholipids extracted from egg yolk and from the liver and spleen of guinea pigs, the fatty acids will inhibit the growth of tubercle bacilli.

Dubos (25) has postulated that the various fatty acids which have been shown to be toxic for tubercle bacilli probably play an important role in the evolution of tuberculous lesions. All of the acids are produced normally, either in the course of tissue metabolism or by autolytic changes following necrosis, and some occur *in vivo* in the range of concentrations which have been found to inhibit bacillary growth *in vitro*. In connection with the present study it is interesting to note the studies by Dubos (25) on bovine- and human-type bacilli. He has shown that greater concentrations of the low molecular weight fatty acids (acetic, propionic, butyric, caproic, caprylic, capric) are required to inhibit growth of the Ravenel strain *in vitro* than are needed to inhibit growth of the human strain H37Rv.

Obviously, it is not possible from the experimental evidence reported herein to attribute the differential effect of the fatty acids toward the human- and bovine-type tubercle bacilli to any single component or group of components contained in the crude mixture. This might have been simply a reflection of technical limitations of the methods used for fractionation. Moreover, it should not be overlooked that all of the acids contained in the mixture might be re-

sponsible for the differential growth inhibition. Studies aimed at the identification of the active component (or components) are in progress in this laboratory.

SUMMARY

The experimental work described in the present paper was undertaken in an attempt to elucidate, if possible, the mechanism underlying the rather high resistance of rabbits to human-type tubercle bacilli (H37Rv) and their extreme susceptibility to the bovine type (Ravenel). Using standard chemical procedures, fractions of normal rabbit lung were prepared which, following removal of protein by alcohol precipitation, were (*a*) water soluble, (*b*) alcohol soluble-acetone insoluble (phospholipids), and (*c*) alcohol soluble-acetone soluble. Whereas the water-soluble and phospholipid fractions were growth supporting for both strains even in relatively high concentrations, the acetone-soluble material was markedly inhibitory, but more so for the human than the bovine organisms.

Subsequent studies suggested that the active components of the acetone-soluble extract were probably fatty acids, since a similar effect could be demonstrated using fatty acids isolated through saponification of either the phospholipid or neutral fat extracted from normal rabbit lung. Separation of the mixed fatty acids from lung phospholipid into volatile, nonvolatile, saturated, and unsaturated fractions failed to reveal that the active material resided in only one component exclusively.

Using rabbit lung phospholipid as a substrate, repeated experiments failed to reveal the presence of phospholipase activity in either culture filtrates or homogeneous suspensions of the H37Rv and Ravenel strains. However, by similar techniques it was found that normal rabbit lung tissue itself is possessed of readily detectable phospholipase activity.

SUMARIO

Los Acidos Grasos de los Tejidos y Su Posible Relación con la Resistencia Natural de los Conejos a la Infección con Bacilos Tuberculosos de Tipo Humano

La labor experimental descrita en este trabajo fué emprendida con mira a dilucidar, si era posible hacerlo, el mecanismo en que se basa la resistencia algo alta de los conejos a los bacilos tuberculosos de tipo humano (H37Rv) y su extremada susceptibilidad al tipo bovino (Ravenel). Usando procedimientos químicos aceptados, preparáronse fracciones de pulmones normales de conejo, las que, a continuación de la separación de la proteína por precipitación con alcohol, eran: (a) solubles en agua, (b) solubles en alcohol-insolubles en acetona (fosfolípidos) y (c) solubles en alcohol-solubles en acetona. En tanto que las fracciones hidrosolubles y fosfolípidas sostenían el desarrollo de ambas cepas aun a concentraciones relativamente altas, la substancia acetono-soluble era decididamente inhibidora, pero más para los microbios humanos que para los bovinos.

Estudios subsiguientes sugirieron que los componentes activos del extracto soluble en acetona eran ácidos grasos, dado que podía observarse un efecto similar usando ácidos grasos aislados por saponificación ya del fosfolípido o de la grasa neutra extraídos del pulmón de conejos normales. La separación de los ácidos grasos mezclados del fosfolípido pulmonar en fracciones volátil, fija, saturada e insaturada no reveló que la substancia activa radicara exclusivamente en un solo componente.

Usando el fosfolípido de pulmón de conejo como substrato, experimentos repetidos no pudieron revelar la presencia de actividad fosfolipásica ni en filtrados de cultivos ni en

suspensiones homogéneas de las cepas H37Rv y Ravenel. Sin embargo, se demostró con técnicas semejantes que el tejido pulmonar de conejo normal mismo está dotado de actividad fosfolipásica que es fácil descubrir.

RESUME

Corrélation possible entre les acides gras tissulaires et la résistance naturelle des lapins à l'infection par le bacille tuberculeux de type humain

Les travaux expérimentaux décrits dans cet article ont été entrepris dans le but d'élucider, si possible, le mécanisme qui préside à la résistance relativement élevée des lapins au bacille tuberculeux humain (H37Rv) et leur extrême sensibilité au bacille de type bovin (Ravenel). Des fractions de poumons de lapins normaux ont été préparés à l'aide de procédés chimiques standard. Après extraction de la protéine par précipitation par l'alcool, ces fractions étaient: a) solubles dans l'eau, b) solubles dans l'alcool et insolubles dans l'acétone (phospholipides), c) solubles dans l'alcool et solubles dans l'acétone. Tandis que les fractions solubles dans l'eau et les phospholipides permettaient la croissance des deux souches, même en concentration relativement forte, la substance soluble dans l'acétone était nettement inhibitrice, et encore plus pour le bacille humain que pour le bacille bovin.

Les études ultérieures permettent de supposer que les constituants actifs de l'extrait soluble dans l'acétone sont probablement des acides gras, car un effet similaire pouvait être démontré en employant des acides gras obtenus par saponification des phospholipides ou des graisses neutres extraites du poumon du lapin normal. La séparation des acides gras mélangés dérivés des phospholipides en fractions volatiles, non volatiles, saturées et non saturées n'ont pas permis de déceler si cette substance active n'existait que dans un seul des constituants.

Des expériences répétées, en utilisant comme substrat le phospholipide du poumon de lapin, n'ont pas permis de déceler une action de la phospholipase, soit dans les filtrats de culture soit dans les suspensions homogènes des souches H37Rv et Ravenel. Toutefois, des techniques similaires ont permis de découvrir que, chez le lapin normal, le tissu pulmonaire lui-même possédait une activité enzymatique (phospholipase) aisément décelable.

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INFECTIVITY OF PULMONARY TUBERCULOSIS IN RELATION TO SPUTUM STATUS[1]

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INTRODUCTION

In his introduction to *Modern Practice in Tuberculosis*, Gloyne (1) rightly draws attention to our comparative ignorance of the stage at which a patient with pulmonary tuberculosis becomes noninfectious. Sufficient information is not available to judge whether a smear negative for acid-fast bacilli on microscopic examination is sufficient or whether it is also necessary to have sputum negative for tubercle bacilli on culture or, in the absence of sputum, cultures of a laryngeal swab or gastric lavage. The investigation presented in this paper attempts to link and assess the degree of risk of infection with the varied sputum status of the patient. For this purpose, source cases of pulmonary tuberculosis which have come under observation during a five-year period have been divided into three groups: (*1*) those with sputum positive for acid-fast bacilli on microscopic examination; (*2*) those with sputum positive for tubercle bacilli only on culture; and (*3*) those with sputum negative for tubercle bacilli by both methods. Whenever possible, the home contacts of these groups have been examined roentgenographically, and the children have been tuberculin-tested. The degree of infectivity of the different groups of source cases has been compared by analyzing the morbidity and tuberculin state of their respective contacts.

Review of Literature

All observers admit the greater liability to infection of home contacts of the "positive sputum" case before diagnosis, compared with those persons not in contact. Opinion varies on the risk after a diagnosis is made, and must depend on facilities for isolation and the care with which these facilities are used. After diagnosis the subsequent tuberculin conversion rate in children has, in certain circumstances, been found to be little in excess of the average rate of conversion for the district under consideration.

The reactor rate of those in contact with so-called "closed" tuberculosis usually has been found to be in excess of that where no contact with tuberculosis exists (2, 3). This rate must depend on the care with which a search is made for tubercle bacilli in the sputum and on whether a genuine "negative sputum" was at some time previously "positive." Blahd and associates (2) found that 9 per cent of noncontact children at the age of twenty-four months were positive to tuberculin and 52.7 per cent of the children who were in contact with "sputum negative" patients (on smear and culture) were tuberculin reactors. Other investigators, however, have reported that the tuberculin state of those in contact with "negative sputum" patients approximated that of those who had never been in known contact with pulmonary tuberculosis (4). It would seem reasonable to expect the latter observation to be

[1] From the service of the North West Metropolitan Regional Hospital Board, London; and the Luton and Bedford Chest Clinics and General Hospitals, England.

more likely if the sputum of the source case were indeed negative for tubercle bacilli.

Cruickshank (5) mentions that the literature on conversion rates and on the risk of developing overt tuberculosis among those in contact with "sputum positive" patients on culture only is scanty. Pottenger (6) and others have stated their opinion that the risk is small. The Scandinavian literature is moderately extensive on the risk of infection from persons with gastric lavage positive for tubercle bacilli on culture.

This literature is well summarized by Bluhm (7), who compared the tuberculin status of those in contact with a "positive sputum" case and those in contact with a "positive gastric lavage" case. He included as "sputum positive" all persons with sputum positive for tubercle bacilli on smear or culture, but failed to separate these two classes. Children in contact with a "positive sputum" case showed a 44 per cent tuberculin conversion rate in the zero-to-five-year age group, whereas the rate of those in contact with "gastric lavage positive" and "gastric lavage negative" cases approximated 6 per cent and was equal in the two classes. No difference was found between these two latter classes and those not in contact with tuberculosis. There was no difference between primary and post-primary lesions in terms of risk created by "positive" gastric lavage. Lassen (8) found 40 secondary cases of tuberculosis among 188 persons in contact with "positive sputum" cases and none among those in contact with 84 "gastric lavage positive" cases.

No mention has been found of tuberculin hypersensitivity and the development of secondary cases among those in contact with either "sputum positive on culture only" cases or those with laryngeal swabs positive for tubercle bacilli

PLAN OF INVESTIGATION

Definitions

Source case. A person discovered to have pulmonary tuberculosis during the period of survey.

Contact. A person who either lived in the same household as a source case or was on very frequent visiting terms.

Sputum positive. Sputum positive for tubercle bacilli on direct smear by the Ziehl-Neelsen technique (ZN), with or without concentration methods.

Sputum positive on culture. This term has been used to indicate:

(*1*) any sputum found negative on direct smear and from which tubercle bacilli have been grown by culture methods only;

(*2*) absence of sputum but tubercle bacilli grown on culture from laryngeal swabs or gastric washings.

Sputum negative. This term indicates that sputum when present was negative for tubercle bacilli on direct smear and culture, and that laryngeal swabs or gastric washings were negative on culture when sputum was absent.

Material and Methods

The survey was carried out in Bedfordshire between January, 1948, and December, 1952. The population during the period was approximately 300,000 persons, roughly divided into thirds: industrial, open town, and rural, respectively.

In order to establish, as far as possible, comparable conditions of contact, a selection of source cases was necessary. All persons with contacts who had previously been in contact

with a known case of tuberculosis were excluded, as such contact might have influenced the tuberculin status and been responsible for the development of secondary cases of tuberculosis. The source cases discovered and satisfying these criteria during the period were divided according to whether the sputum was "positive" (ZN), "positive on culture," or "sputum negative." As a result, there remained 453 source cases with sputum "positive" on direct smear, 230 with sputum "positive on culture," and 321 with "negative sputum," with contacts eligible for examination.

Sputum samples of new patients were submitted to the laboratory over a period in sets of three. Direct smears were examined and all specimens cultured as a routine, if negative. On outpatients this type of sputum series was usually repeated at monthly intervals until a "positive" was found or until at least six, and often twelve or more, specimens had proved negative for tubercle bacilli. After this number, tests were done less frequently. On inpatients, daily tests were usually made for a week in the event of a patient's being "sputum negative" on admission. When no sputum was present, laryngeal swabs were taken in pairs. No case was included in the "culture-positive" group where a single direct "positive" smear was found in the first six months after diagnosis.

Contacts of all three groups were divided into children (under fifteen years) and adults. Only children were tuberculin-tested as a routine. A group of nontuberculous children with no known contact with tuberculosis was included for comparison. These children were selected at random over the period from those attending the chest clinics. All tuberculin tests were done as soon as practicable after diagnosis and, in many instances, before the final sputum status of the source case was established. Whenever there was contact with a "positive" or "culture positive" sputum, children were recalled at three-month intervals. In the event of the occurrence of tuberculin conversion within three months, the results were charted as "positive" but any conversion which occurred after this period was ignored.

Tuberculin testing was performed with tuberculin jelly or Old Tuberculin (OT) intradermally. In younger children, jelly tests were used except when results were doubtful, and then only intradermal tests to 1:100 OT were accepted as negative. In a small number the conditions were not fulfilled, and the results of these children's tests were not included in the survey.

The tuberculin jelly test was performed, using Tuberculin Diagnostic Jelly,[1] in children up to the age of ten years. The jelly was applied after careful cleansing of the skin by ether or acetone and rubbing until an area of erythema was produced. No 'flour paper' was used deliberately as the addition of this technique was found to cause false positives.

Results were read normally on the third day. An area of raised erythema, often with vesiculation, confined to the *V* on which the jelly had been placed, was taken as positive. Doubtful reactions were of two main types: (*1*) those in which the erythema was not sufficiently definite, (*2*) those with a generalized vesicular reaction under the plaster which, although usually indicative of high sensitivity, might have been caused by a plaster reaction.

The intradermal test (Mantoux) was performed with OT in varying strengths up to a maximum of 1:100. These tests were read after forty-eight hours and were only considered positive if an area of induration measuring 5 mm. or more across was present in addition to surrounding erythema.

All contacts of "sputum positive" and "culture positive" source cases were kept under prolonged observation if possible, but contacts of "negative sputum" cases were not normally followed beyond the initial examination unless the sputum status of the source case changed. Secondary cases of tuberculosis occurring among the contacts were included only in the event of confirmed diagnosis. Primary pulmonary tuberculosis in children was only diagnosed in the presence of a roentgenographic abnormality. Enlarged hilar lymph nodes were accepted only if the hilum was seen unequivocally to alter in size while under observation.

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[1] Proprietary name for product of Allen & Hanbury.

OBSERVATIONS

Results of Tuberculin Testing in Children

Three hundred and seventy-four contacts of "sputum positive" cases were tuberculin-tested, and 244 (65.2 per cent) were found to be tuberculin reactors. Two hundred and twenty-eight contacts of "culture positive" cases were tested, and 61 (26.8 per cent) were positive to tuberculin. Two hundred and twenty-one contacts of "sputum negative" cases were tested, and 39 (17.6 per cent) were positive to tuberculin. Seven hundred and nine noncontact children were tested, and 157 (22.1 per cent) were positive to tuberculin. These results are shown in table 1.

In table 2 the results of tuberculin testing in each of these four groups are given in more detail by breaking up the total number of contacts into five-year age classes. The reactor rate of the contacts of the "sputum positive" cases is markedly higher than that of any of the other groups, and this is apparent at all ages, and the degree of difference is significant at all ages. The reactor rates of the other three groups differ very little, although there is a slightly higher number of reactors in each quinquenniad among the "culture positive" contacts. The

TABLE 1

RESULTS OF TUBERCULIN TESTING IN THREE CONTACT GROUPS AND ONE NON-CONTACT GROUP OF CHILDREN AGED 0 TO 14 YEARS

CONTACT GROUP	TOTAL NUMBER TESTED AGE 0–14	REACTORS		NONREACTORS	
		Number	Per Cent	Number	Per Cent
Positive for tubercle bacilli (Ziehl-Neelsen smear)	374	244	65.2	130	34.8
Positive for tubercle bacilli on culture	228	61	26.8	167	73.2
Negative for tubercle bacilli on culture	221	39	17.6	182	82.4
Noncontact group	709	157	22.1	552	77.9

TABLE 2

NUMBERS AND PERCENTAGES OF REACTORS TO TUBERCULIN IN THREE GROUPS OF CHILDREN DIVIDED INTO QUINQUENNIADS

CONTACT GROUP	AGE 0–4 YEARS			AGE 5–9 YEARS			AGE 10–14 YEARS		
	Total	Reactors		Total	Reactors		Total	Reactors	
		Number	Per Cent		Number	Per Cent		Number	Per Cent
Positive for tubercle bacilli (Ziehl-Neelsen smear)	161	97	60.2	114	73	64.0	99	74	74.7
Positive for tubercle bacilli on culture	82	11	13.4	88	27	30.7	58	23	39.7
Negative for tubercle bacilli on culture	83	6	7.2	81	15	18.5	57	18	31.6
Noncontact group	189	12	6.3	297	59	19.9	223	86	38.6

"culture positive" group was also divided into those in contact with sputum "positive" on culture and those in contact only with "positive laryngeal swab" cases. The reactor rate was approximately equal in these last two subdivisions.

The Development of Tuberculosis in Each Contact Group

As previously stated, the three types of contacts were not followed in the same way. Tuberculosis developing in the contacts of the "sputum negative" source cases, unless found on the first examination, would only be discovered after the development of symptoms, and the method of investigation should tend to show less tuberculosis developing in this group. All cases of tuberculosis arising in a contact between January, 1948, and June, 1953, have been included.

Contacts of 453 "sputum positive" source cases: In table 3 it may be seen that, of 669 adult contacts, 73 were diagnosed as having pulmonary tuberculosis; one, tuberculous meningitis; and one, surgical tuberculosis (cervical adenitis). Of 374 child contacts, 42 were diagnosed as having primary pulmonary tuberculosis; and one, post-primary (adult type) pulmonary tuberculosis. Four developed tuberculous meningitis; 6, extrapulmonary tuberculosis of various sorts; and one, miliary tuberculosis.

Contacts of 321 "sputum negative" source cases: In table 3 it may be seen that, of 354 adult contacts, 5 were found with pulmonary tuberculosis. Of 221 children, one was found with primary pulmonary tuberculosis and one with extrapulmonary tuberculosis. Four of the adult cases of tuberculosis were found at first contact examination, and one developed symptoms and was diagnosed later. Both children came under observation for their disease, and the examination of contacts led to the source case.

Contacts of 230 "sputum culture positive" source cases: In table 3 it may be seen that, of 408 adult contacts, 2 cases of pulmonary tuberculosis were diagnosed

TABLE 3

NUMBER OF CASES OF TUBERCULOSIS ARISING AMONG CONTACTS OF THREE TYPES OF SOURCE CASE

NUMBER OF SOURCE CASES	ADULT CONTACTS				CHILD CONTACTS					
		Number with—				Number with—				
	Total	Respiratory Tuberculosis	Tuberculous Meningitis	Other Non-respiratory Tuberculosis	Total	Primary Respiratory Tuberculosis	Post-primary Respiratory Tuberculosis	Tuberculous Meningitis	Miliary Tuberculosis	Other Non-respiratory Tuberculosis
453 sputum-positive	669	73	1	1	374	42	1	4	1	6
230 culture-positive	408	2	—	1	228	4	1	1	—	—
321 sputum-negative*	354	5	—	—	221	1	—	—	—	1

* The "sputum negative" group was examined roentgenographically only once unless the development of symptoms led to further examination. The "sputum positive" and "culture positive" groups were kept under roentgenographic observation throughout the period of the survey.

(one of which was a pleural effusion) and one case of extrapulmonary tuberculosis (cervical adenitis). Of 228 child contacts, 4 were found with primary pulmonary tuberculosis, one with post-primary (adult type) pulmonary tuberculosis, and one with tuberculous meningitis. The 4 persons with primary pulmonary tuberculosis included 2 with pulmonary collapse and 2 with enlarged hilar nodes, one of whom later developed an effusion.

Examination of the contacts of the 3 adults who had tuberculosis led to the discovery of what were judged to be the source cases in 2. Likewise, examination of the contacts of the 6 children with tuberculosis led to the discovery of 5 source cases.

Details of "Culture Positive" Source Cases Associated with Secondary Cases of Tuberculosis

Associated with the 3 adults and 6 children who developed tuberculosis in this group of contacts were 9 source cases. In 6 of these source cases, cavitation was obvious either on the plain film or by tomography. On diagnosis the disease varied in extent roentgenographically from four zones[3] in 2 patients to two zones in 2 patients and one zone in 5 patients.

Tubercle bacilli were obtained from culture of sputum in 8 of the 9 cases, and in one by laryngeal swabbing. The latter case was found after the patient's infant died of tuberculous meningitis; she had four zones of disease with bilateral cavitation.

Of the 230 source cases, material for study was obtained from sputum in 158 (68.7 per cent), from laryngeal swabbing in 66 (28.7 per cent), and from gastric lavage in 6 (2.6 per cent). Active treatment was considered necessary and undertaken in 8 of the 9 source cases.

DISCUSSION

Comparison of the tuberculin sensitivity states of the four groups of children demonstrates a high conversion rate among the contacts of "sputum positive" source cases. This difference is statistically significant and, so far as the "sputum negative" and noncontact groups are concerned, is the result expected and roughly similar to the findings of others.

The conversion rate of the "culture positive" contacts is close to that of the "sputum negative" and noncontacts, but it is slightly higher, especially for the younger age groups. This suggests that the "culture positive" source case may be slightly more infectious than the "negative" source case. No figures for the tuberculin states of "culture positive" contacts have been found in the literature except for the small number reported by Shaw and Wynn-Williams (9). Therefore, comparison with the work of others is not possible.

In regard to the development of tuberculosis, the high over-all morbidity rate of the contacts of the "sputum positive" compared with the "sputum negative" source cases is in conformity with the findings of others. There is no significant

[3] The term "zone" is used here to designate one-third of one lung as seen on postero-anterior roentgenogram.

difference between the "culture positive" and "sputum negative" groups. No earlier figures were found with which to compare the morbidity of "culture positive" with other types of contact. The results of the present investigation suggest, however, that the danger of infection to adults arising from the patient whose sputum is positive for tubercle bacilli only on culture is no greater than that from the patient with "negative" sputum. The occurrence in adults of two cases of respiratory tuberculosis among the "culture positive" contacts and five among the "sputum negative" is presumably due to chance. On the other hand, the discovery of rather more tuberculosis among the child contacts of the "culture positive" source cases is probably evidence of slightly greater infectivity, and is in conformity with the results of tuberculin testing.

Some difference in the degree of infectiousness might be expected between the "laryngeal swab positive" patient with no sputum and the patient with sputum "positive" on culture. The reactor rates of the child contacts in these two subdivisions were equal. However, the numbers involved were small and so no definite conclusion can be drawn. Perhaps it is worth noting that the single contact developing tuberculosis in the "laryngeal swab positive" subdivision was a child with tuberculous meningitis whose mother had gross cavitating bilateral disease, and the failure to find a direct "positive" sputum was surprising.

As the small and doubtful differences in infectivity between the "culture positive" and "sputum negative" groups were found among close family contacts, it is reasonable to assume that they would be much less, if present at all, in the remoter association of those meeting at work. The exclusion of patients with active disease clinically or roentgenographically would render this presumption even more likely. The importance of these findings lies in their application to a policy for the employment of tuberculous patients, who may be regarded as dangerous only in the presence of a sputum positive for acid-fast bacilli on direct smear. A corollary of such a policy must be the careful examination of direct smears by the laboratory, rather than a reliance on culture technique to demonstrate infectivity, as well as frequent serial examinations of sputum to ensure that the patient's status has not changed. From an epidemiologic point of view, it would appear to be quite wrong to classify "sputum positive" and "culture positive" cases together, although it is recognized that from time to time the "culture positive" patient, as indeed the "sputum negative" patient, might in fact be "positive" on direct smear.

SUMMARY

The literature on sputum status and infectivity in pulmonary tuberculosis is briefly reviewed with special reference to the lack of knowledge on the infectiousness of sputum positive for tubercle bacilli only on culture.

Children in contact with "sputum positive on direct smear" cases showed a reactor rate of 65.2 per cent; children in contact with "sputum positive on culture" cases, a rate of 26.8 per cent; and children in contact with "sputum negative on culture" cases, a rate of 17.6 per cent. A group of noncontacts showed a reactor rate of 22.1 per cent.

The morbidity rate of both adults and children was much higher among the "sputum-positive" contacts than among the "culture-positive" and "sputum-negative" groups. The last-named were very much alike, although the balance was slightly in favor of the "sputum-negative" contacts in so far as young children were concerned.

It is concluded that the "sputum-positive" patient (ZN) is much more dangerous to his contacts than the "culture-positive" patient, and that, except possibly in the case of young children, the "culture-positive" patient may be treated in the same way as the "sputum-negative" one from a public health aspect.

SUMARIO

La Variable Infectividad de la Tuberculosis Pulmonar en Relación con el Estado del Esputo

Repásase aquí la literatura relativa a la calidad del esputo y la infectividad en la tuberculosis pulmonar con referencia particular a la falta de conocimientos acerca de la infecciosidad del esputo que sólo resulta positivo en cultivos.

Los niños en contacto con esputo positivo en frotes directos revelaron una tasa de 65.2 por ciento de reactores; con esputo positivo en cultivos, una tasa de 26.8 por ciento; y con esputo negativo en cultivos, una tasa de 17.6 por ciento. Un grupo sin contacto acusó una tasa de 22.1 por ciento de reactores.

Las tasas de morbidad tanto de los niños como de los adultos fué mucho más alta entre los que estuvieron en contacto con esputo positivo que entre los grupos en contacto con cultivos positivos y esputo negativo, los cuales fueron muy semejantes, aunque la diferencia favoreció ligeramente a los contactos con esputo negativo, en lo tocante a niños pequeños.

Dedúcese que el enfermo de esputo positivo (ZN) es mucho más peligroso para sus contactos que el de cultivo positivo y que, con la posible excepción de los niños pequeños, el enfermo de cultivo positivo puede ser tratado del mismo modo que el de esputo negativo desde el punto de vista sanitario.

RESUME

Variations de l'infectiosité de la tuberculose pulmonaire en relation avec l'état de l'expectoration

Les publications sur les rapports entre l'état de l'expectoration et la contagion dans la tuberculose pulmonaire sont brièvement analysées en mettant l'accent sur l'insuffisance des connaissance sur l'infectiosité du crachat B.K. positif en culture seulement.

Chez l'enfant exposé à la contagion, le taux des réactions cutanées positives a été de 65,2 avec le crachat B.K. positif à l'examen direct; de 26,8% pour le crachat positif en culture; et de 17,6% pour le crachat négatif en culture. Un groupe témoin d'enfants non exposés à la contagion donnait un taux de réactions positives de 22,1%.

Les taux de morbidité pour les adultes et les enfants exposés à la contagion étaient beaucoup plus élevés pour les "contacts" crachat B.K. positif que pour les groupes "contacts" culture B.K. positive, crachat B.K. négatif, qui étaient presque semblables. Toutefois, quand il s'agissait de jeunes enfants, le bilan était légèrement en faveur des "contacts" crachat B.K. négatif.

En conclusion, le malade dont les crachats sont positifs (ZN) est beaucoup plus dangereux pour son entourage que le malade dont l'expectoration, négative à l'examen direct, donne des cultures positives. A l'exception des jeunes enfants, le sujet à cultures positives peut être traité de la même manière que la malade à crachats B.K. négatifs, en ce qui concerne l'hygiène publique.

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The writers are much indebted to Dr. J. H. Walker and Dr. W. S. Lane for performing the large number of sputum tests. Their thanks are also due to Dr. W. C. V. Brothwood for his

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TUBERCULIN REACTIONS OF TUBERCULOUS PATIENTS ON ISONIAZID THERAPY[1]

A Preliminary Report

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INTRODUCTION

A decrease in tuberculin sensitivity has been reported in tuberculous guinea pigs under treatment with streptomycin (1, 2). Steenken and Pratt (3), in 1949, found no decrease in tuberculin sensitivity in guinea pigs after twenty-seven to thirty-nine days of streptomycin therapy. Steenken and Wolinsky (4), in 1952, reported a marked decrease in tuberculin sensitivity of guinea pigs with isoniazid therapy.

In humans, Barnwell and co-workers (5) reported no significant difference in tuberculin sensitivity after streptomycin therapy but gave no quantitative data. Woodruff and associates (6) found an increase in tuberculin sensitivity in 34 anergic critically ill tuberculous patients after clinical improvement on streptomycin therapy. From the Veterans Administration Progress Report of June 29, 1952 (7), from Minneapolis, Minnesota, a decrease in tuberculin sensitivity was reported in 13 of 22 patients who had received isoniazid therapy. Criep and Levine (8), in 1953, reported no inhibition of the tuberculin reaction in tuberculous patients after one month of isoniazid therapy.

Before the advent of chemotherapy, changes in tuberculin sensitivity in tuberculous patients were observed. A trend toward decrease in sensitivity with progression of disease and increase in sensitivity with improvement has been reported by Appel and associates (9), Hayes and co-workers (10), Schwartz and Heise (11), and others. There have been many reports in the literature of changes in tuberculin sensitivity due to many varied causes which will not be discussed in the present paper.

It seemed of interest to study changes in tuberculin sensitivity on a quantitative basis in a large number of tuberculous patients on various antituberculous drugs. Through the courtesy of Dr. Esmond Long and Dr. Florence Seibert, a supply of PPD-S was obtained for these studies. This preliminary report gives the results of the tuberculin sensitivity of the entire adult patient group at Dunham Tuberculosis Hospital, Cincinnati, Ohio, and the findings in a small group of patients under isoniazid therapy with repeated tuberculin tests at intervals of one to six months of therapy.

MATERIALS AND METHODS

Tuberculin: PPD-S obtained from Dr. Seibert was used for all tests. This preparation was used because of the possibility of lack of standardization of commercial PPD prepara-

[1] From Dunham Hospital, the Tuberculosis Hospital of Hamilton County, and the Department of Medicine, College of Medicine, University of Cincinnati, Cincinnati, Ohio.

tions. It was also essential that the PPD preparation be constant for a long period of time so that reliable comparison of sensitivity levels could be made. It is of interest that in the course of this study several batches of commercial PPD were checked with the PPD-S by simultaneous testing. It was found that some commercial PPD preparations were much less antigenic than the standard PPD-S, especially in reactions obtained with smaller dilutions. The percentage of reactors was found to be 50 per cent less to the 0.00001 mg. of PPD dilution with one commercial preparation as compared with the standard. Lack of standardization of tuberculin preparations may account for variations obtained in different studies.

The dilutions were made from a stock solution of 0.005 mg. of PPD-S per 0.1 ml. in Buffer No. 2 as the diluent. The formula for the buffer is 4.77 gm. $Na_2HPO_4.12\ H_2O$, 0.363 gm. KH_2PO_4, 8.0 gm. NaCl per liter, sterilized, to which 100 ml. of 5 per cent phenol were added. This stock solution was kept under sterile conditions in a refrigerator and used during a period of six months. The different testing dilutions were made from the stock supply and Buffer No. 2 and used promptly, within one to five days. All dilutions were refrigerated until the actual time of use.

The following dilutions were used in the study:

0.005 mg. of PPD per 0.1 ml. (usual second-strength PPD)
0.001 mg. of PPD per 0.1 ml.
0.0001 mg. of PPD per 0.1 ml. (usual intermediate-strength PPD)
0.00001 mg. of PPD per 0.1 ml. (one-half of usual first-strength PPD)
0.000001 mg. of PPD per 0.1 ml.

All dilutions were made accurately by one technician, and each step was observed and checked by one of the writers. All glassware, pipettes, and syringes were cleansed by boiling for ten minutes in green soap solution, immersion for twenty-four hours in strong sulfuric acid-dichromate cleaning solution, and thorough rinsing with tap and distilled water according to the technique of Nelson, Seibert, and Long (12). New syringes and needles were used in giving the tests.

Testing: All tests were done by the intracutaneous injection of 0.1 ml. of the tuberculin solution on the flexor surface of the forearm and were read at forty-eight hours. All tests and readings were made by the writers, and measurements of the reactions were recorded in millimeters of edema and erythema. In the survey of the hospital patient population, the first test was 0.000001 mg. of PPD. If the reaction was less than 5 × 5 mm. of edema, 0.0001 mg. of PPD was given. In later testing 0.00001 mg. of PPD was used as the second test. Tests larger than 0.0001 mg. were given when no reactions occurred. The maximum dose used was 0.005 mg. of PPD. A positive test was considered to be an area of definite edema measuring 5 × 5 mm. and more at forty-eight hours. Whenever the edema was not dense or was questionable, the next larger test was given.

RESULTS

Tuberculin sensitivity of 483 adult tuberculous patients: The entire adult patient group was tested as an initial study to determine the present general level of tuberculin sensitivity. The distribution of patients in regard to type and extent of tuberculous disease by admission diagnoses was as follows: 36 per cent of the patients were classified as having the pneumonic type of lesion and 64 per cent as having fibroid disease. In 69 per cent, the disease was classified as far advanced; in 25 per cent as moderately advanced; and in 6 per cent of the patients, the disease was classified as minimal. As virtually all patients had received much streptomycin and para-aminosalicylic acid (PAS) therapy and 114 patients had received isoniazid for periods of one to four months, it was thought that the level of tuberculin sensitivity would probably be lower than that found in a previous study at this same institution in 1942.

TABLE 1

NUMBER AND ACCUMULATED PERCENTAGE OF REACTORS TO VARIOUS TEST DOSES OF TUBERCULIN PPD AMONG 483 ADULT TUBERCULOUS PATIENTS BY SEX AND COLOR

SEX AND COLOR	NUMBER TESTED	0.000001 MG. OF PPD				0.0001 MG. OF PPD				0.001 MG. OF PPD				0.005 MG. OF PPD			
		Positive		Negative		Positive		Negative		Positive		Negative		Positive		Negative	
		Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent
White male	211	52	24.6	159	75.4	194	92	17	8.0	208	98.7	3	1.3	210	99.5	1	0.5
Negro male	93	44	47.3	49	52.7	93	100	—	—	—	—	—	—	—	—	—	—
White female	115	26	22.8	89	77.2	109	94.7	6	5.3	112	98.2	3	1.8	114	99.1	1	0.9
Negro female	64	29	45.3	35	54.7	63	98.5	1	1.5	64	100	—	—	—	—	—	—
Totals	483	151	31.3	332	68.7	459	95.0	24	5	477	98.8	6	1.2	481	99.6	2	0.4

In the study in 1942, made before antituberculous chemotherapy, Furcolow, Hewell, and Nelson (13) showed in a quantitative study of tuberculin sensitivity that tuberculous patients were extremely sensitive, 53.4 per cent reacting to 0.000001 mg. and 99.6 per cent reacting to a level as great as 0.0001 mg. of PPD-S. In the present study, it was found that 95 per cent of the patients reacted to this level of 0.0001 mg. Although the standard PPD-S in 1942 and 1952 may not be comparable, it is of interest that tuberculin sensitivity is still high in tuberculous patients despite any effect drug therapy may have on tuberculin sensitivity.

The accumulated percentage of reactors by sex and color of 483 tuberculous adult patients to various test doses of PPD is shown in table 1. Thirty-one and three-tenths per cent of the total group reacted to 0.000001 mg. of PPD, and, in accumulated percentage, 95 per cent of the total group reacted to 0.0001 mg. of PPD.

There is a significant difference in sensitivity of Negro males and females to the 0.000001 mg. test as compared with white males and females. The Negro groups contained more positive reactors to this amount of PPD: 47.3 per cent of the Negro males, compared with 24.6 per cent of the white males; and 45.3 per cent of the Negro females, compared with 22.8 per cent of the white females. In the 1942 study there was no significant difference in tuberculin sensitivity by race and age. There is no ready explanation for the increased tuberculin sensitivity among Negroes unless it reflects a general favorable response to antituberculous drugs. A much higher percentage of Negro than white patients had exudative disease, 62 and 20 per cent, respectively. This type of disease has usually been accompanied by lower tuberculin sensitivity. In the evaluation of clinical improvement of the patients, there was no difference between Negro and white patients.

Tuberculin sensitivity by color, age, and sex is shown in table 2. This emphasizes again that, in the present survey, the Negroes, both males and females, were much more apt to be tuberculin-sensitive than the white patients. There was a striking difference in sensitivity to 0.000001 mg. of PPD in white males more than forty years of age (21.7 per cent positive) and white males less than forty (33.3 per cent). However, the difference was not statistically significant. White females less than forty years of age showed the lowest percentage of positive reactors to 0.000001 mg. From the numbers in this survey, there was no significant difference in tuberculin sensitivity by age in any groups.

As tuberculosis formerly has been considered a disease of younger persons, it is interesting to observe the large number of patients more than forty years of age. This was especially the case among the males, of whom a total of 219 were more than forty, compared with only 86 males less than forty years of age. Fifty-seven per cent of the total group in the study were more than forty years of age, whereas, in the group in 1942, only 23 per cent were more than forty-five years of age.

Comparison of 1952 with 1942 study of tuberculin sensitivity of tuberculous patients: If comparison can be made of the 1952 and 1942 studies in which the

TABLE 2

TUBERCULIN SENSITIVITY IN PER CENT TO VARIOUS TEST DOSES OF PPD BY COLOR, AGE, AND SEX

AGE, SEX, AND COLOR	SENSITIVE TO 0.000001 MG. OF PPD	SENSITIVE TO 0.0001 MG. OF PPD	SENSITIVE TO 0.001 MG. OF PPD	SENSITIVE TO 0.005 MG. OF PPD	INSENSITIVE TO 0.005 MG. OF PPD
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
White males					
Less than 40 (54 patients)	33.3	66.6	—	—	—
More than 40 (157 patients)	21.7	67.5	8.9	1.3	0.6
White females					
Less than 40 (73 patients)	19.2	78.1	—	1.4	1.4
More than 40 (42 patients)	28.6	61.9	7.1	2.4	—
Negro males					
Less than 40 (32 patients)	46.8	53.1	—	—	—
More than 40 (62 patients)	47.5	52.4	—	—	—
Negro females					
Less than 40 (47 patients)	42.6	55.3	2.1	—	—
More than 40 (17 patients)	52.9	47.1	—	—	—

same standard PPD-S was used, the following points are of interest. There was a lower level of sensitivity in the present group to 0.000001 mg. of PPD, 31.3 per cent as compared with 53.4 per cent in 1942. There was also a statistically significant lower level of sensitivity in the present group to 0.0001 mg. of PPD, 95 per cent as compared with 99.6 per cent in 1942. There was a significant difference in sensitivity between Negroes and whites in the present study, a difference not found in 1942. There is a difference in the age groups in the two studies. In the present study, 57 per cent of the group were more than forty years of age in contrast to 23 per cent who were more than forty-five years old in the 1942 study. This difference in age may account for the somewhat lower sensitivity of the whole group.

In comparing the two groups of patients in 1942 and 1952, little difference is noted with respect to type and stage of disease. Fever was much more frequent in 1942, 31 per cent of the patients having low-grade fever and 15 per cent having fever of more than 100° F. at the time of the tuberculin survey. In the 1952 study, only 16 per cent had low-grade fever, and 2 per cent had temperatures higher than 100° F. at the time of the tuberculin testing. Despite the infrequency of fever in the present survey, there is a lower level of tuberculin sensitivity. In the evaluation of the clinical estimate of the progress of the patients, greater improvement is noted in the present study, with 62 per cent improving as compared with 38 per cent in 1942, and 10 per cent becoming worse as compared with 26 per cent in 1942. The evaluations of fever and clinical progress undoubtedly reflect the use of antituberculous drugs.

Analysis of the 24 patients who failed to react to 0.0001 mg. of PPD: As there is interest in lack of sensitivity to tuberculin in patients with proved tuberculosis, the 24 patients who failed to react to 0.0001 mg. of PPD merit comment (tables 1 and 2). Two of the 24 patients were proved not to have tuberculosis. Of the

22 patients with proved tuberculosis, 3 were critically ill and died before tuberculin testing was completed, and one other patient who reacted to 0.001 mg. of PPD also died within a few weeks. The remaining 18 patients reacted to larger doses of PPD as follows: 15 patients to 0.001 mg. of PPD, 2 to 0.005 mg. of PPD, and one failed to react to the 0.005 mg. of PPD dilution. Five of the 18 were febrile at the time of the skin testing. With three others, or 8 in all, they became more sensitive in a few weeks, reacting to 0.0001 mg. of PPD or less. Sixteen of the 18 patients were more than forty years of age; 15 were white males more than forty years old, the group which showed the lowest level of tuberculin sensitivity in those more than forty years old in the hospital population (table 2). The one patient who failed to react to 0.005 mg. of PPD had far advanced disease which was progressing. Four of these patients had received isoniazid therapy for one to four months but without marked improvement. In this group of 22 patients who failed to react to the so-called critical level, 0.0001 mg. of PPD, all had proved tuberculosis and all except one reacted to a level less than the usual second-strength PPD.

There have been recent reports of tuberculin anergy in patients with proved tuberculosis who were not acutely ill or in the terminal stages of their disease. Willi Mascher (14) reported 11 patients with clinical tuberculosis who failed to react to 1 to 3 mg. of tuberculin intradermally. In 6 of the 11, tuberculosis was proved by culture of sputum; in 2, the organisms were found on smear of sputum; and, in 3, the diagnosis could not be confirmed bacteriologically. Arany (15) reported atypical "false positive reactions" in 6 cases of active tuberculosis, that is, reactions at twenty-four hours to large tuberculin concentrations, with negative reactions at forty-eight hours. Lack of tuberculin allergy apparently does occur in tuberculosis but was found by the present writers only in the one case mentioned above.

Tuberculin sensitivity of patients on isoniazid therapy for different periods of time: In table 3 the tuberculin sensitivity is shown of 114 patients of the total 483 originally tested who received isoniazid for periods of one to four months as compared with 369 patients who had received no isoniazid. The majority of the 114 patients were white males and females with long-standing, far advanced disease. There was no significant difference in the tuberculin sensitivity of the isoniazid group as compared with the group which received no isoniazid.

Since the initial survey of all patients, others have been started on isoniazid. A total of 352 persons have been tested after varying periods of therapy. Of 140 patients on isoniazid therapy for two weeks to two months, 74.3 per cent reacted to 0.00001 mg. of PPD, and 86.9 per cent of 212 patients on therapy for two to six months reacted to 0.00001 mg. Thus, there was a trend toward decreased sensitivity after two weeks to two months and a return to greater sensitivity by the third to sixth month of therapy. The difference between the two values is statistically significant. Although this is a significant difference, it would seem that isoniazid in the clinical dosage used (2 to 4 mg. per kg. per day) and for these intervals of time has not produced as striking a change in tuberculin sensitivity as has been observed in animals.

TABLE 3

COMPARISON OF TUBERCULIN SENSITIVITY OF 114 PATIENTS ON ISONIAZID THERAPY FOR ONE TO FOUR MONTHS WITH 369 PATIENTS WHO HAD RECEIVED NO ISONIAZID THERAPY (INITIAL SURVEY)

THERAPY	NUMBER TESTED	0.000001 MG. OF PPD				0.0001 MG. OF PPD				0.001 MG. OF PPD				0.005 MG. OF PPD			
		Positive		Negative		Positive		Negative		Positive		Negative		Positive		Negative	
		Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent
Patients on isoniazid	114	35	30.7	79	69.3	110	96.5	4	3.5	114	100	0	0	114	100	0	0
Patients not on isoniazid	369	115	31.2	254	68.8	349	94.6	20	5.4	363	98.4	6	1.6	367	99.5	2	0.5

In order to determine any slight changes in tuberculin sensitivity, it is important that a definite level of sensitivity be established before therapy is begun in order to provide a reference for subsequent changes in tuberculin sensitivity.

Since the initial survey, therefore, all newly admitted patients have been tuberculin tested with dilutions including the 0.00001 mg. test. This same dilution was used to retest all patients on isoniazid who previously were negative to 0.000001 mg. in order to determine a level between 0.000001 mg. and 0.0001 mg.

In testing tuberculous patients, the three test doses, 0.000001 mg., 0.00001 mg., and 0.0001 mg. of PPD have proved an adequate and safe procedure. Approximately 31 per cent reacted to 0.000001 mg., 68 per cent to 0.00001 mg., and 95 per cent to 0.0001 mg. of PPD. No severe or large skin reactions or systemic ill effects have been observed in repeated tuberculin testing.

Tuberculin sensitivity of 62 patients tested before and repeatedly during isoniazid therapy (2 to 4 mg. per kg.): This is a preliminary report on a small number of patients which includes only those who received a tuberculin test before or within the first two weeks of isoniazid therapy and were retested at one- to two-month intervals. This group is small because of the difficulty of obtaining pretreatment data, as many of the patients received therapy before admission to the hospital. The distribution of the group was 31 white males, 11 white females, 12 Negro males, and 8 Negro females. In this small group there is no apparent difference in change of tuberculin sensitivity by race or sex.

In the present study, a change in sensitivity has been defined as a change in the tuberculin dilution to which the person reacts or as a change of more than 5 mm. of edema in the size of reaction to the same dose. Unless otherwise stated, all changes refer to a change in dilution.

In the group of 62 patients after two months of isoniazid therapy, 30 (approximately 50 per cent) became less sensitive; 16 (approximately 25 per cent) became more sensitive (including one with change in size of reaction to the same dilution); and 16 (approximately 25 per cent) remained stationary. The change in sensitivity of the three groups when tested at one to two and three to five months is shown in figure 1.

Of 30 patients (*Group I*) who were less sensitive than before treatment when tested at one to two months, 28 were retested at three to five months. Eleven remained still less sensitive to tuberculin than before isoniazid therapy and 15 were more sensitive than at two months, showing the same sensitivity as in the pretreatment test. Two were much more sensitive by size of reaction to the same dilution of PPD and are considered to show an increase in tuberculin sensitivity at three to five months. Two have not yet been retested with PPD.

In the 16 patients (*Group II*) whose level of tuberculin sensitivity did not change when tested at two months, 13 were retested at three to five months. Ten of these showed no change in sensitivity, 2 had become more sensitive, and one less sensitive, than before isoniazid treatment.

In the 16 patients (*Group III*) who were more sensitive to tuberculin at two months than before isoniazid was started, 14 were retested at three to five months,

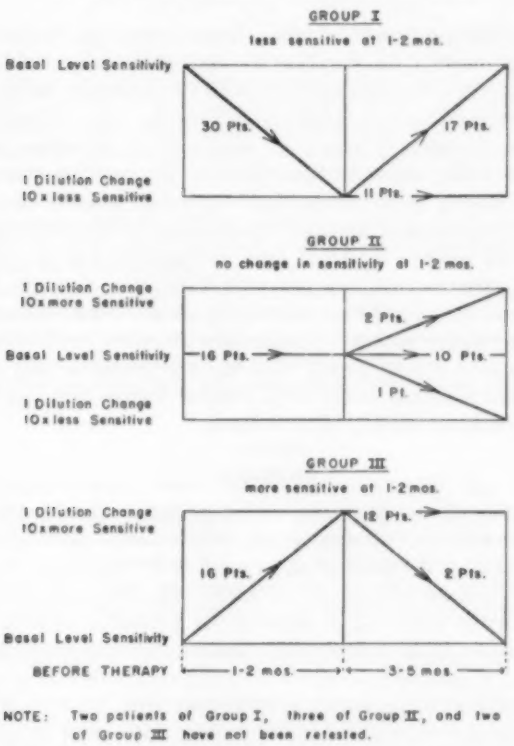

FIG. 1. Changes in tuberculin sensitivity from pretreatment (basal) level at one-to-two and three-to-five month intervals with isoniazid therapy.

and 12 were more sensitive than they were on the pretreatment test. Seven had been febrile or showed other evidence of disease toxicity at the time of the pretreatment test.

To sum up the findings in 62 patients retested one or more times with PPD after the institution of isoniazid therapy: a decrease in tuberculin sensitivity occurred for a short period of one to two months in half of the patients; this decreased tuberculin sensitivity continued for three to five months in only 11; approximately 25 per cent of the patients experienced no change in sensitivity in one to two months; and 25 per cent showed an increase in sensitivity in one to two months. All changes in tuberculin sensitivity represented a positive reaction, i.e., edema of 5 mm. transverse diameter at forty-eight hours, to a dilution ten times greater than that used in the previous test in which a positive reaction was obtained.

A change in tuberculin sensitivity in patients on isoniazid therapy was thus found, but this change has not been of such a degree that tuberculin anergy has developed. All of the 62 patients have reacted to 0.0001 mg. of PPD or to smaller dilutions, all of which are well within the limits of what is considered a positive

tuberculin test. It is not known whether these changes are due to drug therapy alone or possibly occur in the course of tuberculosis untreated with drugs.

An attempt to relate the clinical course with the tuberculin sensitivity in these three groups permitted no conclusions other than that clinical improvement was most definite in the patients who were more acutely ill before treatment and likewise became rapidly more sensitive to tuberculin on chemotherapy.

Tuberculin sensitivity of 25 patients with increased dosage of isoniazid to 10 to 12 mg. per kg.: The dosage of isoniazid was increased from 2 to 4 mg. to 10 to 12 mg. per kg. in 25 patients who had been on isoniazid therapy for five to ten months. These patients were retested with PPD after three to five weeks of increased isoniazid therapy. Twenty-four patients showed no change in the level of tuberculin sensitivity and one became less sensitive. As this group had received isoniazid over a long period of time, it is probable that their tubercle bacilli had become resistant to the drug. Further testing of patients who receive large doses of isoniazid initially will be done.

SUMMARY

The 483 patients who make up the adult population of a tuberculosis hospital were skin-tested with various dilutions of tuberculin (PPD-S). Thirty-one and five-tenths per cent of the total group reacted to 0.000001 mg. of PPD and, in accumulated percentage, 95 per cent to 0.0001 mg., 98.8 per cent to 0.001 mg., and 99.6 per cent to 0.005 mg. A significantly greater number of Negro males and females were sensitive to tuberculin than was the case with white males and females.

Among 352 patients on isoniazid therapy (2 to 4 mg. per kg.), there was a significant trend toward decreased tuberculin sensitivity early in therapy (one to two months) with increased sensitivity later (four to six months). In 62 patients whose status with respect to tuberculin sensitivity was determined prior to isoniazid therapy, 30 showed a decrease in sensitivity after two months, 16 showed no change, and 16 showed increased sensitivity. When the dose of isoniazid was increased to 10 mg. per kg. in 25 patients who had received the drug for five to ten months, there was no change in the level of sensitivity in 24 when they were retested after three to five weeks of the larger dosage.

In this preliminary study with the dosage of isoniazid used and in this group of patients, it appeared that definite changes in tuberculin sensitivity occurred in some patients on isoniazid therapy. No tuberculin anergy has developed, as has been described in animals. These changes in the tuberculin reaction have been well within the limits of what is considered to be a positive reaction to the critical level of tuberculin (0.0001 mg. of PPD).

SUMARIO

Sensibilidad a la Tuberculina de los Tuberculosos Puestos a Isoniacidoterapia: Informe Preliminar

A los 483 enfermos que forman la población adulta de un hospital para tuberculosos se les comprobó cutáneamente con varias diluciones de PPD-S. Un 31.5 por ciento del grupo total reaccionó a 0.000001 mg. y, en porcentajes acumulativos, 95 por ciento a 0.0001 mg.,

98.8 por ciento a 0.001 mg. y 99.6 por ciento a 0.005 mg. Los varones y mujeres de raza negra fueron notablemente más sensibles a la tuberculina que los de raza blanca.

En 352 enfermos sometidos a la isoniacidoterapia (2 a 4 mg. por kg.), hubo una tendencia significativa hacia disminución de la sensibilidad a la tuberculina tempranamente en la terapéutica (uno a dos meses), con aumento después (cuatro a seis meses). De 62 enfermos en quienes se determinó el índice de sensibilidad antes de la isoniacidoterapia, 30 revelaron disminución de la sensibilidad al cabo de dos meses, 16 no mostraron alteración y 16 mostraron aumento de la sensibilidad. Cuando se aumentó la dosis de isoniacida a 10 mg. por kg. en 25 enfermos que habían recibido la droga por espacio de cinco a diez meses, no hubo modificación del índice de sensibilidad en 24 al ser recomprobados al cabo de tres a cinco semanas de recibir las dosis mayores.

En este estudio preliminar con la dosis de isoniacida usada y en este grupo de enfermos, pareció que occurrían alteraciones bien definidas en la sensibilidad a la tuberculina de algunos enfermos en tratamiento con isoniacida. No se ha formado anergia tuberculínica, como la descrita en animales. Esas alteraciones en la reacción a la tuberculina encajan dentro de los límites de lo que pasa por ser una reacción positiva a la dosis crítica de tuberculina (0.0001 mg. de PPD).

RESUME

Sensibilité à la tuberculine du tuberculeux traité par l'isoniazide: Rapport préliminaire

Les 483 malades représentant la population adulte d'un hôpital de la tuberculose ont été soumis aux tests cutanés avec diverses solutions de PPD-S. Sur la totalité du groupe, 31,5 pour cent ont réagi à 0,000001 mg. et, en pourcentage cumulatif, 95 pour cent à 0,0001 mg., 98,8 pour cent à 0,001 mg et 99,6 pour cent à 0,005 mg. Les hommes ou femmes de race noire étaient nettement plus sensibles à la tuberculine que les hommes ou femmes de race blanche.

Chez 352 malades soumis à l'isoniazide-thérapie (2 à 4 mg. par kg), une tendance notable à la réduction de la sensibilité à la tuberculine a été enregistrée dans la période initiale du traitement (un à deux mois) avec augmentation ultérieure de la sensibilité (quatre à six mois). Sur un nombre de 62 malades, dont le degré de sensibilité avait été déterminé avant le traitement par l'isoniazide, 30 ont montré une réduction de la sensibilité au bout de deux mois, 16 aucun changement, et 16 une sensibilité accrue. Quand la dose d'isoniazide a atteint 10 mg. par kg., chez 25 malades soumis au traitement pendant cinq à dix mois, aucune variation du degré de la sensibilité n'a été observée quand de nouveaux tests ont été pratiqués chez 24 d'entre eux, au bout de trois à quatre semaines de traitement avec la dose la plus élevée.

Dans cette étude préliminaire effectuée sur ce groupe de malades avec les doses d'isoniazide mentionnées, on a constaté des variations définies de la sensibilité à la tuberculine chez certains malades traités par l'isoniazide. L'anergie à la tuberculine, telle qu'elle a été décrite chez l'animal n'a pas été observée. Ces modifications de la réaction à la tuberculine se sont maintenues dans les limites de ce qui est considéré comme une réaction positive à la dose critique de la tuberculine (0,0001 mg. PPD).

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DIAPHRAGMATIC PNEUMOCELE COMPLICATING THERAPEUTIC PNEUMOPERITONEUM[1]

A Review and Report of Four Cases

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INTRODUCTION

Therapeutic pneumoperitoneum in the treatment of pulmonary tuberculosis is generally considered to be a relatively innocuous procedure. In the past two decades and especially since the advent of effective chemotherapy, it has been used more and more frequently (1).

Recently, thirty-nine different complications of therapeutic pneumoperitoneum have been listed (2). Among these were rupture of the diaphragm and aggravation or initiation of hernia through the common weak places in the abdominal wall, such as the umbilicus and the inguinal canal. With reference to rupture of the diaphragm, Stein (2) states: "Spontaneous rupture of the diaphragm may occur when there is far advanced disease and an ulcerative process of the diaphragm or if the muscle fibers are sufficiently stretched apart to permit a small rupture of the diaphragm resulting in bleb formation on its pleural surface."

In the present reports, an air-filled sac arising from the diaphragm is designated as a diaphragmatic pneumocele. These sacs are not designated as herniations of peritoneal membrane because they seemed to be caused by a defect in the peritoneal membrane rather than a true herniation.

A diaphragmatic pneumocele may be seen as an annular shadow on the chest film of an asymptomatic person (*Case 3*), or may announce itself dramatically with rupture and development of tension pneumothorax. At this time it may be first seen on a roentgenogram when the lung is completely separated from it by the pneumothorax. In other cases the lesion may be diagnosed at thoracoscopy, with or without prior demonstration of the lesion roentgenographically. The present writers believe that this lesion may be recognized prior to rupture if the characteristic roentgenographic findings are present. Anatomic and physiologic factors in the formation of diaphragmatic pneumoceles are probably similar to factors accounting for the predominance of right-sided pneumothorax associated with loss of air from a therapeutic pneumoperitoneum.

REVIEW OF THE LITERATURE

The earliest mention of diaphragmatic pneumocele was made by Laird (3) in 1945. He described multiple air-filled vesicles on the superior surface of the diaphragm in four patients who were receiving pneumoperitoneum at the time of thoracoscopy. In all of these cases, a phrenic paralysis was present on the side of thoracoscopy. He postulated a defect

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in the peritoneum covering the diaphragm, with stretching and thinning of the latter caused by phrenic paralysis. He did not note the side of thoracoscopy. Laird believed these vesicles would explain the occasional case of air passing through the diaphragm to cause pneumothorax.

Two cases of right-sided pneumothorax associated with loss of air from a therapeutic pneumoperitoneum and the presence of an air-filled sac on the superior surface of the diaphragm have been reported. These cases are almost identical with the first 3 cases in the present report.

The first of the reported cases, that of Yannitelli and associates (4), was published in 1949. A young Negro female developed a right-sided pneumothorax twenty days after pneumoperitoneum refills were discontinued in order to study a cystlike area projected below the right diaphragm. Refills were cautiously reinstituted after a six-week observation period. Pneumothorax then recurred on the right side, this time fatally. At autopsy, an air-filled sac was demonstrated arising between the muscle fibers at the posterolateral insertion of the muscular portion of the diaphragm into the central tendon. Communications from the sac into the peritoneal and pleural cavities were discovered.

The second case, reported by Repa and Jacobson (5), was that of a young white male who developed a ringlike shadow projected below the upper level of the right diaphragm. He then developed pneumothorax on the right side associated with loss of air from his abdomen. Thoracoscopy revealed an air-filled sac in the same location as that of the case reported by Yannitelli and associates (4). There was also a separate tear in the diaphragm. The communication between the peritoneal and pleural spaces was not through the sac.

A possible third case was reported by Williams (6). In that case, prior to the development of a right-sided pneumothorax, roentgenograms showed "two rounded shadows which might be interpreted as air containing bullae." However, neither on thoracoscopy nor on the chest roentgenograms was a diaphragmatic pneumocele demonstrated.

Ross and Farber (7) reviewed the literature on right-sided pneumothorax complicating therapeutic pneumoperitoneum. They reported three cases of their own and twelve collected by questionnaire. They also mentioned a personal communication from Dr. J. Hallam Cope which stated that a bleb on the superior surface of the diaphragm was seen to change in size with respiration, on thoracoscopy. With the exception of two cases thought to be due to congenital perforations of the diaphragm, all pneumothoraces associated with loss of abdominal air were on the right side. One of these exceptions was left-sided (8), and the other was bilateral (9). Both developed pneumothoraces after the initial pneumoperitoneum instillation.

CASE REPORTS

The following cases are reported to illustrate the writers' experience with diaphragmatic pneumocele as a complication of therapeutic pneumoperitoneum and the associated danger of development of tension pneumothorax.

Case 1. F. M., a 24-year-old white male, was admitted to Fitzsimons Army Hospital on August 31, 1951, with the diagnosis of moderately advanced pulmonary tuberculosis of the right upper lobe. In addition to bed rest, he received streptomycin, 2 gm. every third day, and para-aminosalicylic acid, 12 gm. every day, for 120 days, from September 17, 1951, to January 15, 1952. Pneumoperitoneum was instituted on January 4, 1952, with average weekly refills of 1,050 cc. On this regimen, chest roentgenograms showed progressive resolution of the disease until April, 1952, after which time there was only slight shrinkage of the residual infiltrations. In July, 1952, he was promoted to the semi-ambulatory treatment class.

On August 1, 1952, six days after the last refill, he noted the acute onset of right chest pain and shortness of breath. Spontaneous pneumothorax was diagnosed clinically and con-

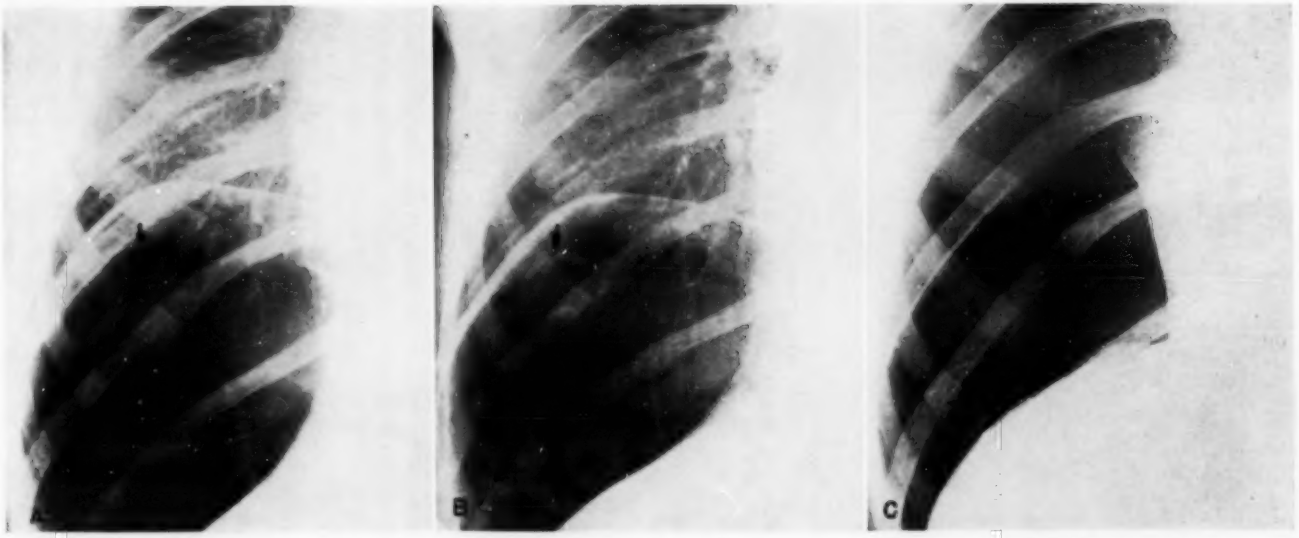

FIG. 1. *Case 1. A.* On the film of July 10, 1952, an annular shadow is seen projected below the right diaphragm in the sixth anterior interspace. *B.* On the film of September 8, 1952, the annular shadow has increased in size and apparently developed loculation. *C.* On the film of November 9, 1952, taken after the occurrence of spontaneous pneumothorax, the diaphragmatic pneumatocele is seen rising above the diaphagrm without connection to the lung. Note the loss of pneumoperitoneal air.

firmed by fluoroscopy. During the following week, fluid was seen in the right costophrenic angle. By August 19, there was complete absorption of air and fluid. Chest films showed no change in comparison with those taken before the incident. Retrospectively, an annular shadow was seen in the film of May, 1952 (figures 1 *A* and *B*). Since May, the lesion increased in size and developed what appeared to be trabeculations. Two pneumoperitoneum refills were omitted in order to observe any change in roentgenographic appearance. With the absorption of air, the diaphragm descended one interspace, and part of the lesion was projected above the diaphragm without change in size. Refills were then reinstituted, and the patient was allowed to progress normally through his treatment classes.

While shaving on the morning of November 9, 1952, five days after the last refill, the patient again noted the sudden onset of chest pain on the right side and shortness of breath. He also experienced a sensation of decrease in abdominal pressure. The pain was a severe aching, referred from front to back at about the level of the diaphragm. When seen at 12:30 p.m. that day, he was dyspneic but not in acute distress. Fluoroscopy revealed right pneumothorax with approximately 15 per cent collapse of the right lung. Because there was a diminished amount of air in the abdominal cavity, it was thought that the air had escaped into the right thoracic cavity. He was seen again at 5:30 that evening because of persistent pain. At this time he was moderately dyspneic and slightly cyanotic. The chest was held in the position of complete inspiration, and a shift of the mediastinum to the left was present. A chest film (figure 1 *C*) confirmed the presence of tension pneumothorax with 80 per cent collapse of the right lung. The lung was adherent at the apex and completely separate from the diaphragm. There was a thin layer of air below the left leaf of the diaphragm. The previously mentioned annular shadow now appeared to be an air-filled sac rising above the diaphragm. A tube was inserted into the pleural space and the lung was quickly re-expanded with water seal drainage. A small pleural effusion was again seen but was absorbed within five days. Subsequent films failed to show the cyst or the pneumoperitoneum.

Just prior to the first pneumothorax, intra-abdominal pressures were plus 14 and plus 20 cm. of water, opening and closing respectively. The patient had four successive refills of 1,200 cc. each prior to the second pneumothorax, and pressures were plus 10 and plus 18 cm. of water, opening and closing respectively. Pneumoperitoneum was discontinued after the second pneumothorax had occurred.

Comment: This was the first case of diaphragmatic pneumocele recognized at this institution, and is chiefly remarkable for illustrating the characteristic features of this lesion. It slowly developed in the posterolateral portion of the right diaphragm. After a premonitory rupture, it ruptured again with the reinstitution of pneumoperitoneum. It was diagnosed by a chest film which revealed the pneumothorax separating the lung from the diaphragm; the pneumocele was definitely located on the diaphragm.

Case 2. L. M., a 19-year-old white male, was admitted to Fitzsimons Army Hospital on February 14, 1952, with the diagnosis of far advanced pulmonary tuberculosis of the right upper and left upper and lower lobes. In addition to bed rest, he began a course of streptomycin, 2 gm. every third day, and isoniazid, 300 mg. each day.

Pneumoperitoneum was initiated on July 3, 1952, and maintained with weekly refills of 750 cc. until December 8, 1952. At this time intra-abdominal pressures were plus 8 and plus 10 cm. of water, opening and closing respectively. In the evening, three days after this last refill, he noted the onset of shortness of breath and some aching in the right chest. A film revealed a right pneumothorax with approximately 60 per cent collapse of the right middle and lower lobes. The right upper lobe was adherent superiorly and posterolaterally to the chest wall. On close examination of this film (figure 2) a 1.5 by 2.0 cm. thin-walled annular shadow was seen rising above the diaphragm, projected behind the anterior portion of the seventh rib. Below the diaphragm, there was a minimal amount of air. Apparently, 90 per

FIG. 2. *Case 2.* Detail of the diaphragmatic pneumatocele rising above the right diaphragm after spontaneous pneumothorax occurred with loss of abdominal air.

cent of the abdominal air had escaped into the right pleural space. The pneumothorax was treated with insertion of a needle and water seal drainage. Recovery was uneventful except for the development of a small pleural effusion which reabsorbed within a week. Pneumoperitoneum treatment was discontinued.

Examination of previous films at this time did not reveal any area suggestive of diaphragmatic pneumocele.

Comment: This is another case illustrating the diagnosis of diaphragmatic pneumocele in the presence of a pneumothorax which separated the lung from the lesion.

Case 3. S. B., a 32-year-old white female, was admitted to Fitzsimons Army Hospital on August 7, 1951, for treatment of moderately advanced pulmonary tuberculosis of both upper lobes and tuberculous pleurisy with effusion. In addition to bed rest, she received streptomycin, 2 gm. every third day, and para-aminosalicylic acid, 12 gm. every day, for 180 days, from June 17, 1951, to January 17, 1952. Pneumoperitoneum was initiated on January 2, 1952. Roentgenographic stability of disease was attained in January, 1952. She was discharged with inactive tuberculosis on July 1, 1952. Further follow-up and pneumoperitoneum refills were given on an outpatient basis.

In June, 1952, a small (0.5 cm.) area of rarefaction appeared projected below the upper level of the diaphragm and behind the anterior portion of the right seventh rib. This area was surrounded by a thin ring without adjacent infiltration. This ring increased slowly in size in the following months, reaching a diameter of approximately 3 cm. by November 18, 1952 (figures 3 *A* and *B*). At this time studies were performed in an effort to rule out a lower lobe tension cavity. Clinically, there were no signs of activity of the tuberculous process.

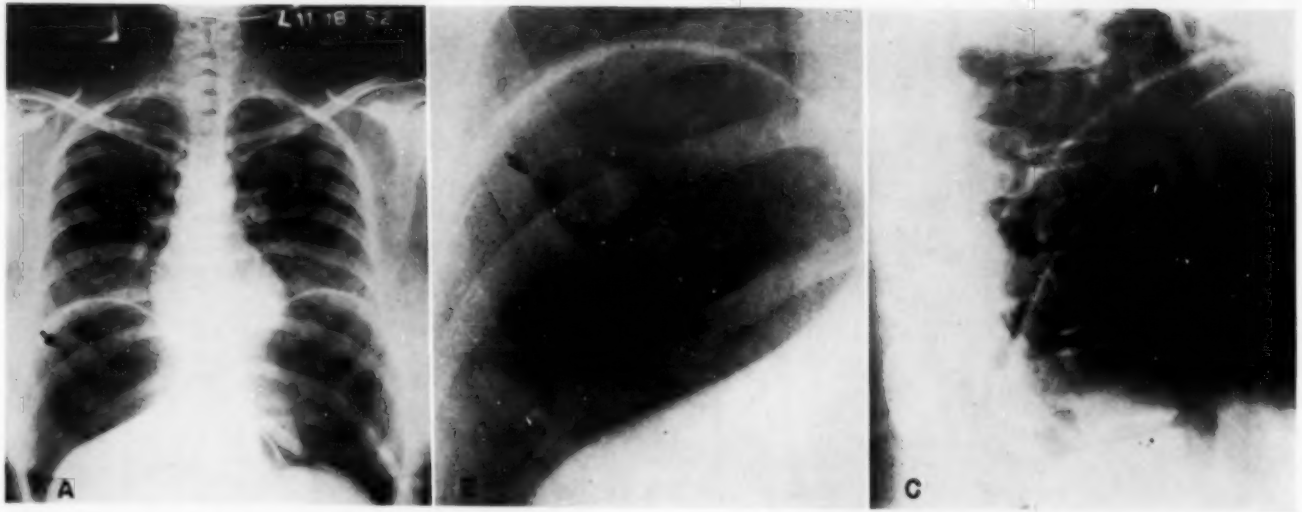

FIG. 3. *Case 3. A.* Chest film of November 18, 1952, showing annular shadow projected below the diaphragm through the sixth and seventh anterior ribs. *B.* Detail of annular shadow, as above. *C.* Lateral view showing C-shaped shadow arising from the posterior portion of the right diaphragm.

Sputum and gastric washings were negative for tubercle bacilli. Bronchoscopy revealed normal findings.

It was then decided that this was a diaphragmatic pneumocele. A lateral film (figure 3 *C*) showed it to be continuous with the posterior arc of the right leaf of the diaphragm. Pneumoperitoneum was therefore discontinued and, with the absorption of the abdominal air, the pneumocele disappeared.

Comment: This lesion was recognized by its characteristic roentgenographic appearance and course. The diagnosis was confirmed by the disappearance of the lesion when pneumoperitoneum was discontinued and the abdominal air had absorbed.

Case 4. M. W., a 31-year-old white male, was admitted to Fitzsimons Army Hospital on September 23, 1951, for treatment of moderately advanced pulmonary tuberculosis of both upper lobes. In addition to bed rest, he received streptomycin, 2 gm. every third day, and para-aminosalicylic acid, 12 gm. daily, from September 28, 1951, to May 4, 1952. Therapeutic pneumoperitoneum was initiated on April 8, 1952, and continued with weekly refills of 750 cc. until January 20, 1953.

In October, 1952, the patient began to complain of pain in the right upper abdomen after each refill. The pain was severe enough to require codeine for relief the first evening, and usually lasted approximately twenty-four hours after each refill.

On January 20, 1953, the patient received his usual refill. The next evening he began to complain of sharp, constant pain in the right upper abdomen. Approximately thirty minutes later he noted more severe abdominal pain radiating up into the right shoulder and the right side of his neck. At the same time he noted a sensation of decreased tension in his abdomen and some shortness of breath. Fluoroscopy and a chest roentgenogram (figures 4 *A* and *B*) at this time revealed a pneumothorax with 20 per cent collapse of the base of the right lung. The upper portion of the lung was adherent to the chest wall. There was a decrease in the amount of air in the abdomen. A slight shift of the mediastinum to the left was present. In the left anterior oblique position, a small cystlike shadow was seen on the anterior portion of the right diaphragm, approximately 5 cm. from the mediastinum and close to the anterior chest wall. This lesion increased in size on inspiration and appeared to have a somewhat thicker wall than the previously described pneumoceles.

A needle aspiration of the chest was performed, removing 1,200 cc. of air from the right pleural cavity. Pressures on completion of this procedure were minus 9 and minus 13 cm. of water, expiratory and inspiratory, respectively. Fluoroscopy after removal of air revealed almost complete re-expansion of the lung. Two more aspirations of air were performed within the next two weeks. A small amount of fluid was noted in the right costophrenic angle the day after the onset of the pneumothorax and this persisted for approximately three weeks. Following discontinuation of pneumoperitoneum refills and absorption of all abdominal air, the pneumocele was not seen in subsequent chest films.

Comment: This case is unusual in that the location of the pneumocele was anterior. There are two possible explanations for this unusual location. This could represent a hernia of the Foramen of Morgagni (figure 5) inflated and perforated by pneumoperitoneum, or it could be a diaphragmatic pneumocele protruding between muscle bundles of the anterior part of the diaphragm.

Since it is hard to fit this case into the theory of pathogenesis to be discussed later, it is believed this represents a hernia through the Foramen of Morgagni (10). To explain the origin of this lesion on the same basis as that of the first three cases, it would be necessary to implicate the coronary ligament of the liver. This latter explanation seems more tenuous than considering the lesion to be an inflated hernia through the Foramen of Morgagni.

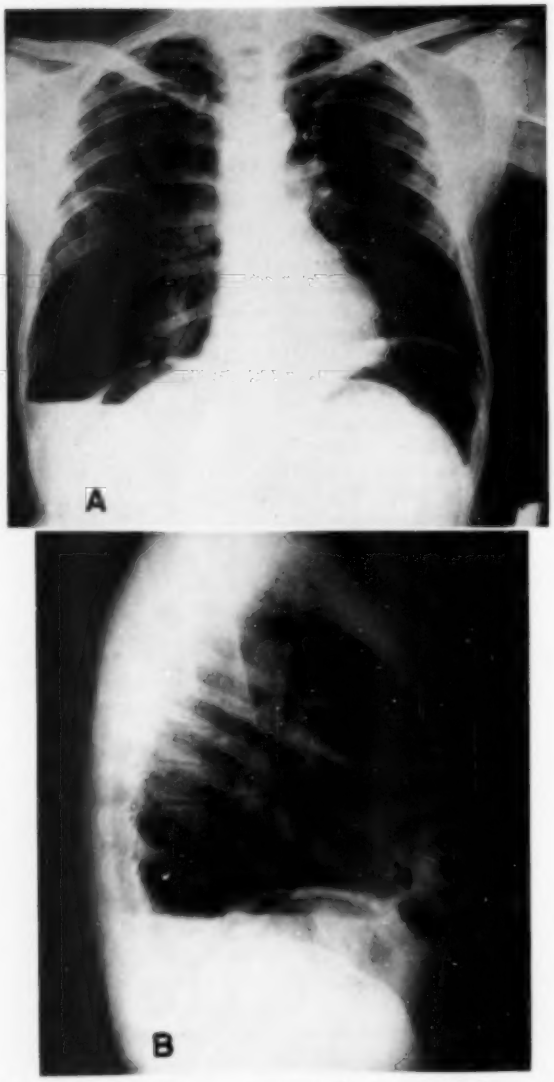

FIG. 4. *Case 4. A*. Chest film of January 27, 1953, showing right hydropneumothorax with a small amount of residual abdominal air. The air-filled sac is seen projected below the right diaphragm. *B*. Lateral view showing the air-filled sac just behind the sternum.

FIG. 5. Location of common hernia of the diaphragm. (*From Thoracic Surgery Course, Fitzsimons Army Hospital, 1953*)

PREVALENCE OF DIAPHRAGMATIC PNEUMOCELES

The chest films of 500 patients who were receiving therapeutic pneumoperitoneum were reviewed. Two additional patients were found to have had diaphragmatic pneumoceles. This represents an incidence of 1.2 per cent (6 cases in 500). However, there were 3 cases of right-sided pneumothorax complicating therapeutic pneumoperitoneum in which diaphragmatic pneumoceles were never demonstrated in the available chest films.

DISCUSSION

All pneumothoraces complicating pneumoperitoneum, which are attributed to rupture of the diaphragm, are on the right side (7). There must be anatomic and physiologic reasons for this weakness of the right side of the diaphragm. A good explanation should consider the dynamic changes which occur with the institution of pneumoperitoneum. Upon establishment of the therapeutic pneumoperitoneum, the most striking change is separation of the liver from the diaphragm.

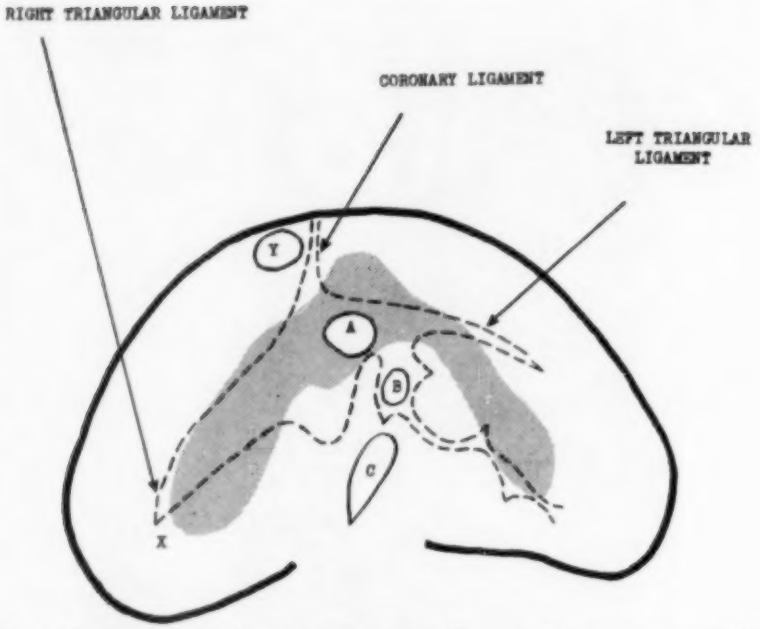

FIG. 6. Diagram of the diaphragm seen from below. The central tendon is represented by the hatched area. Mesenteric attachments are outlined by an interrupted line. *A*, *B*, and *C* are the hiatuses for the inferior vena cava, esophagus, and aorta, respectively. The approximate location of the lesion in *Cases 1*, *2*, and *3* is marked by an *X*. The outline of the lesion in *Case 4* is marked by a *Y*. (*Based on Arey and Grant*)

The liver is then supported below by resting on the remainder of the abdominal viscera, and from above by its attachments to the diaphragm through the right and left triangular ligaments, the coronary ligament, and the attachments of the liver to the inferior vena cava through the hepatic veins (figure 6). In its descent the liver rotates its superior and lateral surfaces downward and toward the midline. In so doing, increased tensions are exerted in an abnormal direction on the diaphragm, especially through the right triangular ligament. This abnormal tension may separate some fibers of the diaphragm at the posterior and lateral attachment of the triangular ligament.

The defect could then be exploited by the net pressure difference between the peritoneal and pleural spaces. Anderson (11) has shown that immediately after refill the pressures of the abdominal and pleural cavities are increased. Afterwards, the pressures decrease, the intrapleural pressure decreasing to nearly its normal negative state while the intra-abdominal pressure decreases but remains positive. At this point, it may be noted that the normal subdiaphragmatic pressure is negative in the erect position, but is positive after introduction of air into the peritoneal space. This means that there is an increase in the net pressure

across the diaphragm after the institution of pneumoperitoneum. The inspiratory movement of the diaphragm also increases the pressure difference between the pleural and peritoneal spaces.

These same factors may be at work in those cases of rupture of the diaphragm without formation of a pneumocele. It also seems reasonable that formation of a pneumocele is evidence of a more chronic process and possibly has a protective function, tending to decrease the tendency of the diaphragm to rupture.

There are two reports describing the histologic features of diaphragmatic pneumoceles. A small sac with communications to the pleural and peritoneal cavities was found at autopsy in the case reported by Yannitelli and associates (4). Microscopically, the sac was composed of fibrous and areolar tissue covered on both sides by granulation tissue. A biopsy of the sac in the case of Repa and Jacobson (5) revealed "connective tissue and muscle fibers compatible with normal diaphragmatic tissue." In neither case was the lesion referred to as a hernia. It is difficult to evaluate the possibility of this lesion's being a hernia. To some extent it depends on what definition of hernia is used. However, there is no statement which indicates that all layers of the diaphragm are involved as components of these sacs. The present writers prefer to believe that a small tear in the peritoneal membrane over the diaphragm allows air to rise between muscle fibers. Possibly at first, the wall of the sac is composed of fibrous and areolar tissue with an upper covering of pleural mesothelium. Later, this upper surface is rubbed by the lower surface of the lung and becomes covered by granulation tissue.

The roentgenographic findings are characteristic but not pathognomonic of diaphragmatic pneumocele. The lesion first appears during the course of a therapeutic pneumoperitoneum as a small area of radiolucency with a complete surrounding ring (annular shadow) projected below the diaphragm. During the next five to six months it enlarges slowly, possibly reaching a diameter of 5 cm. With the increase in size, most lesions develop apparent trabeculation. The size may vary with existing differences of the pleural and abdominal pressures, although the general trend is one of constant slow enlargement. On a lateral film the pneumocele will be seen to arise from the posterior curve of the diaphragm. Regardless of the angle from which the film is taken, the lesion cannot be separated from the diaphragm. Finally, diagnostic or spontaneous pneumothorax will, with separation of the lung from the diaphragm, confirm the origin of the pneumocele.

The criterion for the diagnosis of diaphragmatic pneumocele is demonstration of an air-filled sac which arises from the right leaf of the diaphragm during the course of therapeutic pneumoperitoneum. With this in mind, it is easy to exclude such things as herniation of omentum through a patent Foramen of Bochdalek (12) or annular shadows at the base of the lung which existed before or without pneumoperitoneum. The Foramen of Bochdalek (figure 5) is most often found patent and on the left side (13, 14). Herniation through the Foramen of Morgagni or inflation of such a hernia may be very difficult to differentiate from the usual diaphragmatic pneumocele, except by position (*Case 4.*). Basal lesions of the lung

such as cavities, abscesses, and bullae are readily demonstrable by different projections of chest roentgenograms.

Differentiation of causes of rupture of the diaphragm associated with pneumothorax and loss of air from a therapeutic pneumoperitoneum is essentially differentiation between a tear in the diaphragm and perforation through a diaphragmatic pneumocele. This differentiation is not possible unless the defect is demonstrated in some way, preferably by thoracoscopy. Although a pneumocele may be demonstrable by chest roentgenography, this is not sufficient proof that the perforation occurred through it. This is confirmed by the case reported by Repa and Jacobson (5), in which thoracoscopy demonstrated a tear in the diaphragm separate from the pneumocele.

Differentiation of the resulting pneumothorax is made easier by the universal appearance of pneumothorax on the right side when it can be attributed to rupture of the diaphragm with therapeutic pneumoperitoneum. In contrast, pneumothorax due to either mediastinal emphysema or spontaneous rupture of the esophagus (15) occurs most frequently on the left side. In addition, mediastinal emphysema or rupture of the esophagus is not associated with noticeable loss of abdominal air. Friedman (16) has reported inflation of an esophageal hiatus hernia by pneumoperitoneum, but rupture of such a hernia has not been reported to date. In the event of such a hernia's perforating into the pleural cavity, it would be expected to cause a left pneumothorax due to the anatomic relations of the esophageal hiatus.

Recognition of a diaphragmatic pneumocele is believed to be a contraindication to continuing pneumoperitoneum therapy, since in four known cases this was followed by rupture of the diaphragm. Pneumoperitoneum therapy is also contraindicated if air from the abdomen has entered the thorax. This last may be a relative contraindication since two cases are noted by Ross and Farber (7) as having right pneumothoraces associated with loss of abdominal air and later having pneumoperitoneum successfully reinstituted. In one, the accidental pneumothorax was continued therapeutically until pleural symphysis took place, at which time pneumoperitoneum was again successfully used. In the other, the lung apparently became adherent to the defect in the diaphragm. In all other reported cases of rupture of the diaphragm associated with pneumoperitoneum, reinstitution of pneumoperitoneum was followed by a recurrence of the right-sided pneumothorax. In one case, noted by Ross and Farber (7), pneumoperitoneum was attempted unsuccessfully five times before turning to other methods of treatment.

Until the air is almost completely absorbed, there is danger of rupture of the abdominal air into the pleural space. The patient in the report by Yannitelli and associates (4) developed a pneumothorax twenty days after pneumoperitoneum refills were discontinued. To explain this, it must be remembered that the period after refill is one of increasing pressure difference between the peritoneal and pleural cavities. Previously, the refills may have been given before the critical pressure for rupture was reached.

Simple needle aspiration of the pneumothorax is frequently adequate treat-

ment of this complication. Aspirations can be performed as frequently as necessary to keep the patient comfortable and to speed the expansion of the lung. Inserting a tube into the pleural space and maintaining water seal drainage is not usually necessary. Objections to the latter procedure are mainly the comfort of the patient, the increase in nursing care necessary for water seal drainage, and the attendant danger of contamination of the pleural space.

The pleural effusion which occurred in *Cases 1*, *2*, and *4* disappeared shortly after the pneumothorax air was absorbed and required no special treatment.

SUMMARY

Four cases of a diaphragmatic pneumocele are discussed, and the pertinent literature is reviewed.

This lesion is a small air-filled sac arising from the right diaphragm during the course of therapeutic pneumoperitoneum. It is associated with danger of perforation of the diaphragm and development of tension pneumothorax with loss of peritoneal air.

An attempt is made to explain the development of the pneumocele. Implicated in this explanation are changes of the pressures in the peritoneal and pleural cavities and abnormal tensions exerted by the right triangular ligament of the liver on the diaphragm in the presence of therapeutic pneumoperitoneum. It is also suggested that the Foramen of Morgagni is a separate and additional site of weakness which may lead to pneumothorax.

SUMARIO

Neumocele Diafragmático como Complicación del Neumoperitoneo Terapéutico: Repaso de la Literatura y Presentación de Cuatro Casos

Al discutir 4 casos de neumocele diafragmático, repásase la literatura pertinente.

La lesión consiste en un pequeño saco lleno de aire que se forma en la porción derecha del diafragma durante el neumoperitoneo terapéutico. Lleva consigo el peligro de perforación del diafragma y de aparición de neumotórax valvular con pérdida del aire peritoneal.

Discútense la incidencia, el diagnóstico diferencial, el aspecto radiográfico, la anatomía patológica y el tratamiento del neumocele diafragmático.

Trátase de explicar la formación del neumocele. En exta explicación figuran alteraciones de la presión en las cavidades peritoneal y pleural y tensiones anormales ejercidas por el ligamento triangular derecho del hígado sobre el diafragma cuando existe un neumoperitoneo terapéutico. También se apunta que el agujero de Morgagni es otro sitio independiente de debilidad que puede producir neumotórax.

RESUME

Pneumocèle diaphragmatique compliquant un pneumopéritoine thérapeutique: Analyse et observations de quatre cas

Quatre cas de pneumocèle diaphragmatique sont étudiés et la littérature s'y rapportant est analysée.

Cette lésion consiste en une petite poche remplie d'air qui se forme au niveau du diaphragme droit au cours du pneumopéritoine thérapeutique. Le pneumocèle entraîne le risque d'une perforation diaphragmatique avec développement d'un pneumothorax avec pression et perte de l'air péritonéal.

La fréquence, le diagnostic différentiel, l'aspect radiologique, la pathologie et le traitement du pneumocèle diaphragmatique sont étudiés.

Les auteurs tentent d'expliquer le mode de développement du pneumocèle. La variation des pressions dans les cavités pleurales et péritonéales et la tension anormale exercée par le ligament triangulaire droit du foie sur le diaphragme en présence du pneumopéritoine thérapeutique sont considérés dans l'explication de ce phénomène. Le foramen de Morgagni est également suggéré comme lien de moindre résistance additionnel pouvant conduire à la formation d'un pneumothorax.

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SEVERE REACTIONS OF THE CENTRAL NERVOUS SYSTEM FOLLOWING ISONIAZID TREATMENT[1]

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INTRODUCTION

The first communications about the treatment of tuberculosis with isoniazid and iproniazid, stating the relative harmlessness of these compounds, already indicated the possibility of toxic side effects on the nervous system (1–3). The possibility of these toxic manifestations was confirmed by further clinical and experimental studies (4–9). These reactions consisted of vasomotor vertigo, muscular twitchings, hyper-reflexia, delay in micturition, constipation, euphoria, headaches, numbness and parasthesias, dryness of the mouth, somnolence, excessive dreaming, excitation, restlessness, polyneuritis (10, 16), et cetera.

Experimental studies confirmed the possibility of toxic effect of isoniazid and iproniazid on the nervous system, both central and autonomic, which, in toxic doses, caused in experimental animals stimulation of central nervous system, excitement, prostration, running movements, and convulsions. It was pointed out that in clinical application these toxic side effects on the nervous system are more apt to occur from the use of iproniazid than from isoniazid. Moreover, the reactions are more likely in persons with unstable personality, in the older age groups, after administration of general anesthesia, and in previously psychotic persons (4). The last statement was not confirmed by the study of the effect of isoniazid on 51 mentally ill tuberculous patients, who showed improvement in the mental behavior in a reasonably high percentage of cases and no untoward reactions (11).

With the further accumulation of experience with isoniazid, the possibility of more serious complications of the central nervous system was noted. The "withdrawal syndrome" upon discontinuance of iproniazid and isoniazid therapy was described. This occurred only after prolonged treatment, and consisted of insomnia, vertigo, weakness, irritability, nightmares, depression, and drowsiness. These symptoms may last from one to several weeks after discontinuation of treatment (7). A report of the Committee on Chemotherapy and Antibiotics (American College of Chest Physicians) (12) mentioned the potential toxic effect of isoniazid on the central nervous system and urged caution in using this drug in epileptics. The report of the Committee on Therapy (American Trudeau Society) (13) emphasized the frequency of central nervous system effects of isoniazid treatment, some of which may be serious; for instance, acute psychoses of the manic type. A case of death in an epileptic was reported following treatment with isoniazid (14); also cases of acute psychoses following isoniazid treatment with persecution and paranoid ideas, and instances of visual, olfactory, and auditory hallucinations were reported recently (10, 15, 16).

Since the introduction of isoniazid treatment at this Hospital, the writers have seen 5 cases of severe reaction involving the central nervous system following isoniazid treatment, and it is believed that the report of these cases may be of interest for the study of toxicology of isoniazid and related compounds.

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[1] From the Molly Stark Hospital, Canton, Ohio.

CASE REPORTS

Case 1 (Reg. No. 4509). The patient, J. K., a 49-year-old white male laborer, was admitted to Molly Stark Hospital on July 7, 1951, with bilateral far advanced pulmonary tuberculosis. The sputum was positive for *Mycobacterium tuberculosis*; other laboratory tests were noncontributory. The patient weighed 130 pounds. There was no personal or family history of mental and nervous condition of any kind, no history of alcoholism.

Pneumoperitoneum and right pneumothorax in addition to streptomycin-para-aminosalicylic acid (PAS) were ineffectual and on April 25, 1952, a course of isoniazid, 200 mg. daily (on experimental basis at that time), without streptomycin was given for four months without apparent effect.

On January 20, 1953, a course of isoniazid (same dosage) was resumed in combination with intermittent streptomycin. On March 6, 1953, the patient, who had always been a cooperative and pleasant man, started showing signs of nervous irritability, became argumentative, and refused to answer questions. Within the next days he developed a profound depression, refused to take his meals, complained of headaches. After a few days he became a little more cheerful and cooperative, but occasionally cried. Isoniazid was stopped on March 28, 1953.

On March 29, the patient became irrational and mentally confused, threatened to commit suicide, escaped from his room and was caught on the porch. Under the effect of sedation, the symptoms gradually subsided and, on April 4, 1953, the patient seemed to be completely recovered. The isoniazid-streptomycin treatment was resumed after three weeks under "cover" of barbiturates and has been continued up to date without untoward effect.

Case 2 (Reg. No. 4495). The patient, J. V., a 65-year-old white male, retired foundry worker, was admitted to Molly Stark Hospital on May 19, 1951, with history of known pulmonary tuberculosis for the last three weeks and history of silicosis since 1945. There was no personal or family history of mental disorders, "spells" of any kind, or of alcoholism. The sputum was positive for *Mycobacterium tuberculosis*, the other laboratory tests were noncontributory, and the patient weighed 110 pounds. The treatment consisted of bed rest and general supportive measures until January 13, 1953, when isoniazid (200 mg. daily) with routine intermittent streptomycin was started.

On April 8, 1953, the patient was noted to be very nervous, talking to himself and going to other rooms, talking incoherently to other patients. A change of personality also was noted; the patient, a very quiet and cooperative patient hitherto, became irritable and unbalanced. He developed insomnia and profound depression, and appeared to be irrational at times. The symptoms gradually increased and, on May 5, he became completely irrational and restless, and attacks of anger alternated with bursts of tears. PAS was substituted for isoniazid on May 11, 1953. The psychotic symptoms gradually subsided and the patient returned to his normal status on May 20, 1953. At the time of writing this paper (June 23, 1953), the patient appears to be completely normal and rational. Isoniazid treatment was not resumed.

Case 3 (Reg. No. 4583). The patient, H. S., a 57-year-old, white female housewife, was admitted to Molly Stark Hospital on April 12, 1952, because of excavating tuberculoma over the left upper lobe. The sputum was positive for *Mycobacterium tuberculosis*, the blood sugar ranged between 100 and 150 mg. per 100 cc. The patient was known to have a mild diabetes, well under control. Other laboratory tests were noncontributory. The patient's past history and family history were noncontributory at the time of admission, but on retrospective history (obtained from her relatives) "nervousness" throughout life and a history of rather unstable and emotional personality could be revealed. Nevertheless, no real psychotic spells or mental distress could be traced in the past.

During the hospital course, the patient was put on a diabetic diet, general supportive measures, and insulin, which was discontinued on October 20, 1952. On July 6, 1952, the pa-

tient had a left segmental resection (Dr. E. B. Kay) and streptomycin-PAS treatment was initiated and continued up to November 7, 1952, when isoniazid was substituted for the PAS in a dosage of 200 mg. daily. The patient weighed 145 pounds at that time.

The patient continued to feel well and showed no abnormalities in her behaviour until the first part of February, 1953, when a gradual change of her personality was noted. She became very nervous and depressed, often cried, became uncooperative, refusing to take food and medication at times. She gradually developed persecution ideas, became scared of thieves, and thought somebody was after her, following and watching her constantly. She occasionally had "lucid" periods when she acted in a normal manner. Sedation was started and isoniazid continued. Finally, on March 3, 1953, her symptoms became aggravated, she became mentally confused, refused to take food and medication, developed visual and auditory hallucinations. Isoniazid was discontinued on March 6, 1953, but no improvement was noted. She became worse and, on March 20, 1953, attempted suicide by drowning in the bath tub. She also developed strong erotic emotions with hallucinations and started writing love letters to doctors. Her family took her home on March 22, 1953. She continued to behave abnormally while at home and finally was commited to Massillon State Hospital, where she is under treatment at the present time.

Case 4 (Reg. No. 4464). The patient, S. P., a 31-year-old Negro male steel-pourer, was admitted to Molly Stark Hospital on February 12, 1951, with history of known tuberculosis since March, 1950. His sputum was positive for *M. tuberculosis* on admission, other laboratory tests were noncontributory; he weighed 124 pounds. There was no personal or family history of mental disease or severe alcoholism. Nevertheless, it was found on retrospective history that the patient always was a rather peculiar fellow, seclusive, unsociable, and nontalkative, but never had any "spells" of any kind. He received two courses of streptomycin-PAS, which resulted in deafness. Right pneumothorax was started on August 1, 1951, and abandoned prior to a left pneumonectomy, which was performed (Dr. E. B. Kay) on December 6, 1952. A new streptomycin-PAS course was started at that time.

The postoperative course was entirely uneventful, but, on December 20 and 21, the patient became restless and appeared to be confused at times. After December 22, he completely recovered and showed no abnormal symptoms whatsoever. On January 30, 1953, PAS therapy was changed to isoniazid, 200 mg. daily. On February 4, 1953, left thoracoplasty was done, and the postoperative course was uneventful.

On March 7, 1953, the patient became uncooperative, but otherwise acted as usual. On March 23 the patient became restless, mentally confused, and irrational, occasionally disoriented as to time and place. PAS was substituted for the isoniazid, and the patient was sedated. The acute symptoms subsided after March 27, but his mental condition steadily deteriorated. He became highly depressed, stopped talking and answering questions, developed persecution ideas (information obtained through his sister), and occasionally refused to take food. Finally he stopped taking food and fluids and had to be fed by gastric tube for some time. He continues to be highly depressed, does not speak at all, and occasionally refuses to take food. The question of transferring him to a mental institution is being considered.

The following case illustrates the hazard of isoniazid treatment in epileptics:

Case 5 (Reg. No. 4615). The patient, S. T., a 29-year-old white female, was admitted to Molly Stark Hospital on July 21, 1952, with history of known pulmonary tuberculosis since June, 1952. The sputum was positive for *M. tuberculosis* on admission.

The patient, a friendly, but mentally retarded and physically well-developed girl, weighing 124 pounds, presented also a history of epilepsy since her childhood. Until the age of twenty-one she had had frequent epileptic attacks, but since she reached twenty-two years of age they have been diminishing in frequency and severity and she was free of seizures for two years prior to her admission to Molly Stark Hospital. On the day of admission, however,

while in the hospital hall, she developed weakness and tremor which were interpreted as a mild epileptic attack. Her treatment in the hospital consisted of bed rest, general supportive measures, sedation, and streptomycin-PAS-isoniazid treatment, 200 mg. daily, started July 31, 1952.

On August 30, 1952, a light epileptic attack occurred. She remained very nervous on the following day. A mild attack occurred on September 9, 1952. After the attack she remained very nervous; cried. On the next day another attack occurred at 7 a.m. The patient received phenobarbital (60 mg. three times daily) in addition to her regular Dilantin (100 mg. three times daily). Another attack occurred on the same evening, lasting twenty minutes.

Isoniazid was discontinued on October 20, 1952. Under increased sedation, the patient felt relatively well (but was depressed, nervous, and cried occasionally) until October 26, 1952, when she had five seizures with heavy generalized convulsions. After each seizure her emotional disturbance increased. On the following three days, the patient had daily seizures several times a day, lasting from ten to thirty minutes. In the period between the attacks, the patient was semiconscious and had to be fed parenterally. Between October 30 and November 18 the patient had only occasional short and relatively mild seizures, but developed a profound and lasting depression. On November 19 she developed numerous and almost continuous attacks, accompanied by severe generalized clonic convulsions. She sank into coma, her breathing became stertorous, temperature rose to 103°F. The same situation was present throughout November 20 and 21. On November 20, forty-nine attacks were registered by the nurses. The patient received chloral hydrate rectally and was put on sodium phenobarbital intravenously (up to 1.9 gm. within twenty-four hours).

On November 22, the patient started to improve; the severity and frequency of seizures diminished; but she remained unconscious. On November 23 she regained consciousness, started voiding and taking fluids, but remained extremely weak and exhausted. Sedation was gradually reduced and brought to the October dosage. On November 26, the patient apparently had recovered, took food and medication. On December 15, a severe tremor of body and extremities was noted, but no seizure occurred. She had one mild seizure lasting ten minutes on December 17, others on December 22 and 23; tremor and mild convulsions without loss of consciousness occurred on January 21, 1953. The last attack, mild and short, was registered on March 1, 1953. At the present time (June 23, 1953) the patient is in good condition and seems to be emotionally undisturbed.

COMMENT

The possibility of a psychotic reaction as a manifestation of toxic effect of isoniazid on the central nervous system has been mentioned by the Committee on Therapy, American Trudeau Society (13), and cases of psychosis following isoniazid treatment have been described in the literature (10, 15, 16).

In the present *Cases 1* and *2*, psychotic symptoms occurred in previously mentally healthy persons with no history of unstable personality or neuropathy of any kind. The appearance of psychosis was unusual in these patients and, since there were no other known factors which might have contributed to the development of psychosis and since psychotic symptoms gradually disappeared after discontinuance of isoniazid treatment, it is believed that the treatment was a causative factor in the mental disturbance.

Cases 3 and *4* represent the action of isoniazid in persons of emotional, unstable, and psychopathic constitution. Nevertheless, the patient of *Case 3* had reached the age of fifty-seven years without ever having any "spells" or emotional distress of serious nature. In *Case 4* there was a "spell" immediately after surgery (following general anesthesia?) before isoniazid was started. This was of short duration (two days) and the patient showed a complete recovery at that time.

It was pointed out (4) that, in persons with unstable personality or previous psychotics, the central nervous system reactions are more apt to develop. It is believed that isoniazid in *Cases 3* and *4* might have acted exactly in such a way, representing a "trigger mecha-

nism" in the already labile nervous system or at least representing a contributory factor. There are indications (10) that prolonged isoniazid treatment (over a month) may cause anatomic changes in the nervous system. If this is true, it might explain the continuation of symptoms after withdrawal of the drug.

Case 5 is similar to the case previously published (14) with the difference that the present patient survived in spite of extremely severe attacks and continuous convulsions. At no previous time in her life had she had such terrible attacks and she had not had a single attack within two years prior to her admission to the hospital. There is no other explanation for the extreme severity of the seizures, and it is firmly believed that isoniazid was the only responsible factor in this case, causing a violent reaction of a diseased central nervous system. The fact that the second severe attack of convulsions occurred a month after discontinuation of isoniazid does not diminish the belief in the toxic effect of the drug in this case. In fact, action of isoniazid ("withdrawal syndrome" (7)) may persist weeks after discontinuance of treatment. In this case the first severe seizures occurred while the patient was still under treatment, and then persisted in a less severe form until November 19 when, a month after discontinuance, they reached a climax in a rather dramatic fashion.

It has been reported that the toxic action of isoniazid may be diminished by use of barbiturates (4, 14, 17). From this point of view, it is rather interesting to notice that, in the epileptic case, seizures developed, the patient being under sedation, but were checked by a massive intravenous sedation. It is believed that the intravenous injections of sodium phenobarbital in large doses might have been responsible for saving the patient's life.

Another interesting point is a low dosage of isoniazid in all of the present cases, 3 to 4 mg. per kg. daily, which indicates the possibility of a toxic effect of isoniazid even in small doses. This may be a very significant fact and should be considered by those who advocate high therapeutic doses of isoniazid despite the fact that antimicrobial action of isoniazid apparently does not increase with the increase of its dosage after having reached a certain concentration (18).

It is also interesting to note that the length of time between the beginning of isoniazid treatment and the appearance of complications was rather short, being approximately one and one-half months in *Cases 1*, *4* and *5*; three months in *Cases 2* and *3*. The observation was made (10) that approximately 8 per cent of patients given isoniazid for more than a month are liable to develop lesions of the nervous system. In each of the present cases, more than a month elapsed before the development of complications and, in a case previously published (14), the time was much shorter. It also is worth emphasizing that the complication occurred with the use of the less toxic isoniazid, not iproniazid.

In spite of low toxicity, isoniazid may occasionally give rise to serious complications of the nervous system even when given in low doses and for a relatively short time. In some cases these complications are unpredictable; in some, their possibility should be borne in mind when dealing with unstable personalities or patients with a history of mental or nervous disorders of any kind. Special caution is required in epileptics. Barbiturates in reasonably high doses should be given simultaneously in every case to counteract the toxic effect of isoniazid where the possibility of nervous or psychotic reaction might be expected. Having in mind that higher incidence of complications should be expected with higher doses of isoniazid, the optimal minimal dosage should not be exceeded in the suspicious cases. The studies of mode of action of isoniazid (18), showing that the antimicrobial action of this drug does not increase with the increase of its concentration, seem to justify this statement.

The possibility of severe complications should be kept in mind by those who prefer

to treat tuberculosis with isoniazid in the office or patient's home instead of sending him to a hospital or sanatorium.

SUMMARY

Five cases of severe reactions of the nervous system following isoniazid treatment are reported. Four of these reactions were of psychotic type; one in an epileptic consisted of extremely severe epileptic seizures, accompanied by coma of three days' duration. It is believed that, in the 2 cases of psychosis and in the case of epilepsy, isoniazid was the only and direct cause of complications; in another 2 cases of psychosis, isoniazid might have represented a contributory cause or a "trigger action." The role of barbiturates for counteracting the toxic effects of isoniazid on the nervous system has been stressed.

SUMARIO

Graves Reacciones del Sistema Nervioso Central Consecutivamente a la Isoniacidoterapia

Preséntanse 5 casos de reacctiones graves del sistema nervioso a continuación de la isoniacidoterapia. Cuatro de esas reacciones fueron de forma psicótica y una en un epiléptico consistió en accesos epilépticos sumamente graves, estado convulsivo y coma de tres días de duración. Parece que en 2 casos de psicosis y en el caso de epilepsia, la isoniacida fué la causa única y directa de las complicaciones, en los otros 2 casos de psicosis, la droga tal vez representara una causa contribuyente o "acción desencadenante." Recálcase el papel de los barbitúricos para contrarrestar los efectos tóxicos de la isoniacida sobre el sistema nervioso.

RESUME

Réactions sévères du système nerveux central consécutives au traitement par l'isoniazide

Cinq cas de réactions sévères observées à la suite d'un traitement par l'isoniazide sont rapportés. On a observé quatre réactions psychotiques et une du type épileptique avec crises comitiales sévères et état convulsif avec coma qui a duré trois jours. Les auteurs pensent que dans deux cas de psychose et dans le cas d'épilepsie l'isoniazide était la cause unique directe des complications; dans les deux autres cas de psychose, l'isoniazide pourrait avoir eu une action de déclic (trigger action). Le rôle des barbiturates comme antagonistes des effets toxiques de l'isoniazide sur le système nerveux est mis en évidence.

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BCG VACCINATION IN SILICOSIS[1, 2]

II. An Experimental Study of the Influence of Inhaled Quartz Dust upon Infection by BCG (Aronson), H37Ra, and *M. Marinum* Strains of Tubercle Bacilli

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INTRODUCTION

Previous studies (1) demonstrated that BCG organisms of the particular strain Rosenthal 826L, injected intracutaneously into guinea pigs whose local tissue resistance has been modified by inhaled quartz dust, will spread from the site of inoculation, localize in the lung and the tracheobronchial lymph nodes, and produce progressive and often fatal tuberculosis.

The present report describes a second series of experimental studies to determine whether two other strains of tubercle bacilli, an attenuated strain and an avirulent strain, will produce progressive tuberculosis when the bacilli are injected intracutaneously into guinea pigs which had inhaled quartz dust. In addition, a strain of mycobacteria pathogenic for cold-blooded animals was included in the experiments in order to ascertain whether inhaled quartz dust would also alter the native tissue immunity of a warm-blooded animal, such as the guinea pig, to that organism.

MATERIALS AND METHODS

The strains of tubercle bacilli selected for the studies were as follows:

The attenuated[5] bovine tubercle bacillus BCG strain 873 was sent by Dr. J. D. Aronson of the Henry Phipps Institute, and the authors are greatly indebted to him for his cooperation. Dr. Aronson obtained the strain in 1949 directly from the Pasteur Institute, where it had been isolated and carried on glycerolated-water-potato medium for sixteen generations. At that time the strain was tested for virulence by inoculating 10 mg. intratesticularly into guinea pigs; at autopsy three months later, the animals showed no evidence of progressive tuberculosis.

The avirulent *Mycobacterium tuberculosis* variant H37Ra was dissociated (2) from the human tubercle bacillus H37 which was isolated in 1905 by Dr. E. R. Baldwin from the sputum of a patient with chronic pulmonary tuberculosis. The strain was obtained from the Trudeau Laboratory and subsequently was carried in the Saranac Laboratory. Tests for virulence have consistently failed to demonstrate that this dissociant will produce tuberculosis in normal guinea pigs.

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[1] From the Saranac Laboratory of the Trudeau Foundation, Saranac Lake, New York.

[2] The BCG and H37Ra studies were supported by a research grant from the Committee on Medical Research of the American Trudeau Society, medical section of the National Tuberculosis Association; the *M. Marinum* studies were supported by a James Alexander Miller Fellowship.

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[5] In view of the results of this investigation and of the previous study (1), it seems best to refer to the BCG organism as "attenuated" rather than "avirulent."

The *Mycobacterium marinum* strain (3) was also sent by Dr. Aronson, who isolated the organism from tubercles discovered in various organs of salt water fish. The organism is pathogenic for pigeons, mice, goldfish, and frogs, but does not kill rabbits or guinea pigs.

The techniques employed were described in detail in the previous publication (1) but are summarized here for completeness of this report.

Preparation of bacillary suspension: Cultures of each strain maintained on glycerolated-water-potato medium were transferred to Sauton liquid medium on two successive occasions. The twelve-day-old surface bacterial film growth of the second transplant of each strain was collected and used to prepare the fresh vaccine. After pressing the collected yield between pads of blotting paper to remove excess moisture, a weighed amount of the dried bacilli was triturated in a diluent consisting of one part of Sauton medium and three parts of 0.85 per cent sodium chloride solution. Sufficient diluent was added to make a final concentration of 1 mg. of bacilli per ml. All procedures were carried out under sterile conditions and every precaution to avoid contamination was observed.

Infection of animals: A volume of 0.1 ml. of the suspension containing 0.1 mg. of the respective strain of organism was injected intracutaneously into the left flank of each guinea pig. For each of the strains used the injections were made from the same suspension and on the same day.

Exposure to dust: The inhalation of quartz dust by the animals followed in general the routine established by the Laboratory for the production of experimental silicosis (4). In accordance with that routine, guinea pigs lived in a dust room in which, for eight hours daily and six days a week, an atmospheric cloud of quartz dust was maintained by a mechanically operated dust machine. The concentration of the dust was checked by periodic examination of atmospheric samples collected with the Greenberg-Smith midget impinger apparatus and counted by the light-field microscopic technique (5, 6). Study of the samples disclosed that the atmospheric concentration of the dust was approximately 500 million particles per cubic foot of air. More than 99 per cent of the atmospheric particles were less than 10 microns in diameter and thus were sufficiently small to be inhaled into the alveoli of the lung. Chemical analysis of the dust samples revealed a total silica content of 99.3 per cent; this entire amount was free silica in the form of quartz.

Tuberculin skin tests: These tests were performed on all guinea pigs prior to the intracutaneous infection and again at intervals of 78 days and 295 days on those surviving after that infection. Each animal received intracutaneously 0.1 ml. of a 3 per cent suspension of Old Tuberculin. Reactions were read at forty-eight hours and were classified 1-plus when the edema and induration in any diameter were less than 10 mm., 2-plus when the edema and induration were greater than 10 mm., 3-plus when an area of central ischemia accompanied the induration, and 4-plus when ulceration occurred.

Preparation of tissue for study: At intervals during the course of the experimental investigation, animals died from natural causes or were killed for study. Their organs, especially the lungs and the tracheobronchial lymph nodes, were examined grossly. Subsequently, specimens from the lungs and from the nodes were selected for bacteriologic studies, the remaining tissue being preserved for histopathologic studies. For the histologic examination, the lung was fixed by the intratracheal injection of Zenker's fluid. The lung was then sectioned from apex to base in a plane following the course of the trachea and major bronchi, thus furnishing whole sectional mounts for study of the histopathology and for correlation with the anatomic relationships.

Isolation, subculture, and virulence tests of the infecting organisms present in the tissue: Cultures for tubercle bacilli were made from the lungs and from the tracheobronchial lymph nodes of most of the animals. To isolate the organisms, separate portions of the lungs and of the nodes from each animal were crushed in a sterile mortar with a small amount of coarse sterile sand in physiologic saline. The mixture was transferred to a test tube and allowed to stand for five minutes to permit the sand and crushed tissue to settle. The supernatant fluid was withdrawn, digested with an equal quantity of 4 per cent sodium hydroxide

solution at 30°C. for thirty minutes, and centrifuged at 3,000 r.p.m. for ten minutes. The sediment was then neutralized with hydrochloric acid and seeded on three tubes of the American Trudeau Society potato-egg medium. A sterile technique was followed at all times. Cultures were retained for two months before they were considered negative. Virulence tests were carried out by injecting 10 mg. of the culture growth subcutaneously into the right groin of each of 4 healthy adult guinea pigs. These animals were killed for observation at 30, 60, 120, and 240 days after inoculation.

EXPERIMENTAL RESULTS

Experiment I: BCG Infection Occurring Simultaneously with a Developing Silicosis

A group of 25 healthy adult guinea pigs (tables 1 and 4) were infected intracutaneously with the BCG strain of tubercle bacilli, and immediately thereafter their exposure to quartz dust by inhalation was started. By the eighth to the tenth day each pig had developed an indurated nodular lesion at the intracutaneous site. The lesions varied from 5 to 8 mm. in diameter and from 1 to 2 mm. in thickness. By the fourteenth day a small superficial scaling with ulceration of the skin over the lesions was noted, but there was no deep necrosis, caseation, or discharge. By sixty days the lesions were completely healed, leaving in most animals a small, barely detectable scar.

Tuberculin skin tests were made on all animals alive at 78 days and again on those surviving at 295 days after infection. Initially, the reactions were strongly positive, most animals showing ischemia or ulceration at the site of injection; later, the reactions were generally less intense and in most cases had dropped one or two categories in the index scale and showed only induration. This was true even in those animals (numbers 49, 50, 52, and 53) which at autopsy exhibited advanced tuberculosis complicating the silicotic process in the lungs.

During the course of the observations for a period of 608 days, 20 of the pigs were killed and 5 died from natural causes. Two of the 5 died early from a complicating nontuberculous pneumonia, and the other 3 from a progressive tuberculosilicosis. In animals killed at 62 and 127 days, the draining inguinal and iliac nodes on the injected side were slightly to moderately enlarged. At 181 days and thereafter the nodes were small and appeared normal and were no different from those on the contralateral side.

At post-mortem examination (figures 1 to 8), tuberculosis complicating the silicotic process was found in 13 of the 25 pigs, being confined to the tracheobronchial lymph nodes in 9 pigs and involving the lungs and nodes in 4 pigs. In the latter, the infectious component of the pulmonary disease was first observed after 423 days and had progressed to cavitation in one pig at 557 days after the intracutaneous infection. The disease was fatal in 3 pigs. The last 2 animals of this experiment were killed 608 days after infection; they failed to show gross or microscopic evidence of tuberculosis.

Tubercle bacilli were isolated by culture from the tracheobronchial lymph nodes in 12 of the pigs. The lungs from only 16 pigs were studied bacteriologically, and 5 of these gave positive cultures. It is worth noting here that bacilli were isolated from the bronchial nodes as early as 62 days after intracutaneous infection. Later, after 423 days, positive cultures were obtained from the lungs and nodes in only those animals with progressive tuberculous disease.

TABLE 1

EXPERIMENT I

INTRACUTANEOUS BCG (ARONSON) AND QUARTZ INHALATION SIMULTANEOUSLY

GUINEA PIG NUMBER	DATE OF INFECTION	TUBERCULIN SKIN TEST		MODE OF DEATH	TIME AFTER INFECTION	TUBERCULOSIS		ORGANISMS ISOLATED	
						Lungs	Tracheobronchial Lymph Nodes	Lungs	Tracheobronchial Lymph Nodes
		January 24, 1951	August 29, 1951		*days*				
31	November 7, 1950	—	—	K	62	0	NS	*	0
32	November 7, 1950	—	—	K	62	0	NS	*	P
38	November 7, 1950	3+	—	K	127	0	0	*	P
45	November 7, 1950	3+	—	K	127	0	0	*	P
37	November 7, 1950	4+	—	K	181	0	0	0	0
46	November 7, 1950	4+	—	K	181	0	0	0	0
47	November 7, 1950	4+	—	DPn	191	0	NS	*	0
48	November 7, 1950	3+	—	DPn	191	0	NS	*	0
33	November 7, 1950	3+	—	K	241	0	0	0	0
34	November 7, 1950	3+	—	K	241	0	NS	*	P
35	November 7, 1950	4+	3+	K	304	0	NS	P	P
36	November 7, 1950	3+	2+	K	304	0	NS	P	P
39	November 7, 1950	3+	2+	K	364	0	IS	*	P
40	November 7, 1950	3+	2+	K	364	0	IS	*	P
49	November 7, 1950	3+	2+	D	423	CO	IS	P	P
41	November 7, 1950	4+	2+	K	426	0	0	0	0
42	November 7, 1950	3+	1+	K	426	0	0	0	0
43	November 7, 1950	4+	2+	K	489	0	0	0	0
44	November 7, 1950	3+	2+	K	489	0	0	0	0
50	November 7, 1950	4+	3+	K	547	CO	IS	0	P
55	November 7, 1950	3+	2+	K	547	0	0	0	0
53	November 7, 1950	4+	2+	D	557	CA	IS	P	P
52	November 7, 1950	4+	2+	D	581	CO	CO	P	P
51	November 7, 1950	3+	2+	K	608	0	0	0	0
54	November 7, 1950	3+	3+	K	608	0	0	0	0

Key

Tuberculin Test:

- — Not done
- 1+ Induration < 10 mm. diameter
- 2+ Induration > 10 mm. diameter
- 3+ Induration with ischemia
- 4+ Superficial ulceration

- K Killed for sampling
- D Died
- Pn Nontuberculous pneumonia
- * No bacteriologic studies
- P Positive for *M. tuberculosis*

Tuberculosis:

- 0 No recognizable tubercles; no positive cultures
- NS Recognizable nonspreading tubercles
- IS Isolated spreading tubercles
- CO Isolated spreading tubercles with coalescence
- CA Extensive areas of coalescent tuberculous lesions with cavitation

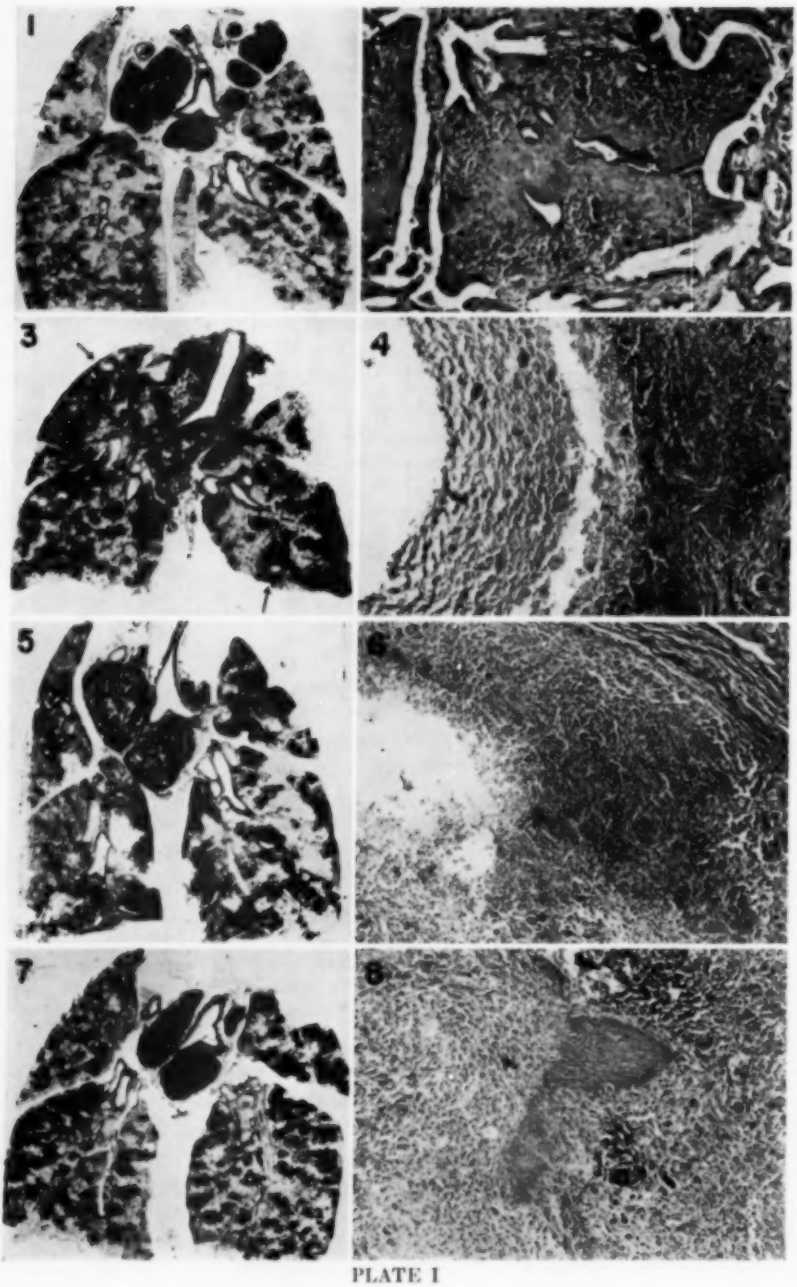

PLATE I

Experiment II: BCG Infection Superimposed upon an Established Silicosis

Twenty-five guinea pigs (tables 2 and 4), exposed for 120 days to quartz dust by inhalation, were removed from the dust room and immediately were infected intracutaneously with aliquot portions of the same suspension of BCG used in the previous experiment. On the next day the animals were returned to the dust room and their exposure to quartz dust was continued. Observations pertaining to the intracutaneous site of inoculation and to the tuberculin skin tests were not unlike those recorded for *Experiment I*. There were minor differences but, in general, the cutaneous lesions ultimately healed, leaving a faint residual scar. The initial tuberculin reactions elicited in the animals comprising the group ranged from 2-plus to 4-plus. The variations persisted in animals tested for the second time and, as was noted in the previous experiment, the reaction was less intense in certain animals even though they ultimately died with cavitary tuberculosis.

At post-mortem examination (figures 9 to 14), tuberculosis was detected in the tracheobronchial lymph nodes of 20 pigs and was observed microscopically as early as 62 days after the intracutaneous infection. The first indication of

PLATE I

FIG. 1. *Experiment I*. Guinea Pig Number 55. Intracutaneous BCG (Aronson) and inhalation of quartz dust. Killed at 547 days. The gross section exhibits the scattered irregular lesions of silicosis in the lung and the silicotic enlargement of the tracheobronchial lymph nodes. There is no evidence of complicating tuberculosis. (1.5 ×)

FIG. 2. Photomicrograph of the silicotic reaction in the lung shown in figure 1. The lesion is irregular, producing thickening of the alveolar walls and obliteration of the air spaces. Mature acellular hyalinized material comprises the central area of the reaction. (200 ×)

FIG. 3. *Experiment I*. Guinea Pig Number 53. Intracutaneous BCG (Aronson) and inhalation of quartz dust. Died at 557 days. The gross section reveals the coalesced lesions of tuberculosilicosis which replace the greater portion of the lung. Scattered small focal areas of liquefaction necrosis may be visualized in the lung and particularly in the enlarged nodes about the trachea. Small cavities are seen in the apex of the upper lobe on the left and at the base of the lower lobe on the right. (1.5 ×)

FIG. 4. Photomicrograph of the cavity wall in the left upper lobe shown in figure 3. Note the inner pyogenic membrane resting upon an outer zone of tuberculosilicotic tissue on the right. (200 ×)

FIG. 5. *Experiment I*. Guinea Pig Number 52. Intracutaneous BCG (Aronson) and inhalation of quartz dust. Poor condition, and died at 581 days. The gross section reveals the confluent tuberculosis involving the lung and enlarged tracheobronchial lymph nodes. (1.5 ×)

FIG. 6. Photomicrograph of an area of tuberculosilicosis with liquefaction necrosis in the tracheobronchial lymph nodes seen in figure 5. The central area of necrosis is encroaching upon the surrounding tuberculosilicotic tissue. (200 ×)

FIG. 7. *Experiment I*. Guinea Pig Number 54. Intracutaneous BCG (Aronson) and inhalation of quartz dust. Killed at 608 days. The gross section shows scattered irregular silicotic lesions, some of which have merged with one another. The tracheobronchial lymph nodes are enlarged. Tuberculosis was not detected. (1.5 ×)

FIG. 8. Photomicrograph of the reaction to inhaled quartz dust deposited in a tracheobronchial lymph node from the animal of figure 7. The reaction is one with a central irregular area of mature acellular hyalinized fibrous tissue surrounded by a few lymphocytes and a thick compact zone of large mononuclear cells. Definite histologic evidence of tuberculosis cannot be detected. (200 ×)

TABLE 2

EXPERIMENT II

QUARTZ INHALATION FOR 120 DAYS, THEN INTRACUTANEOUS BCG (ARONSON) WITH QUARTZ INHALATION CONTINUED

GUINEA PIG NUMBER	DATE OF INFECTION	TUBERCULIN SKIN TEST		MODE OF DEATH	TIME AFTER INFECTION	TUBERCULOSIS		ORGANISMS ISOLATED	
						Lungs	Tracheobronchial Lymph Nodes	Lungs	Tracheobronchial Lymph Nodes
		January 24, 1951	August 29, 1951		*days*				
4	November 7, 1950	—	—	DPn	47	0	0	*	*
3	November 7, 1950	—	—	K	62	0	NS	*	P
27	November 7, 1950	—	—	K	62	0	NS	*	P
12	November 7, 1950	3+	—	DPn	90	0	NS	*	*
8	November 7, 1950	3+	—	DPn	97	0	NS	*	*
5	November 7, 1950	3+	—	K	127	0	0	*	P
9	November 7, 1950	3+	—	K	127	0	0	*	P
6	November 7, 1950	4+	—	K	181	0	NS	0	P
7	November 7, 1950	2+	—	K	181	CO	NS	P	P
23	November 7, 1950	3+	—	D	191	IS	NS	*	P
24	November 7, 1950	3+	—	D	191	NS	NS	*	P
1	November 7, 1950	3+	—	K	241	IS	NS	P	P
2	November 7, 1950	2+	—	K	241	IS	IS	P	P
10	November 7, 1950	3+	2+	K	304	0	NS	0	P
11	November 7, 1950	3+	2+	K	304	0	0	0	0
21	November 7, 1950	3+	2+	D	363	CA	CO	P	P
22	November 7, 1950	4+	4+	K	364	CO	CO	P	P
13	November 7, 1950	3+	3+	K	426	IS	IS	P	P
14	November 7, 1950	4+	2+	K	426	0	0	0	P
17	November 7, 1950	3+	3+	D	430	CO	IS	P	P
19	November 7, 1950	3+	3+	D	430	IS	IS	P	P
20	November 7, 1950	3+	2+	D	433	CO	IS	P	P
26	November 7, 1950	2+	2+	D	448	CO	IS	P	P
18	November 7, 1950	3+	2+	D	456	CA	IS	P	P
25	November 7, 1950	3+	2+	D	475	CO	IS	P	P

Key: *see* Table 1.

spreading tuberculosis in the lungs was found in one animal killed at 181 days. Subsequently, the pulmonary infection was noted in the majority of the animals (13 of 16 animals) and in 2 of these it had progressed to cavitation. During this time more animals died from natural causes than were killed. None survived longer than 475 days after infection. Tubercle bacilli were isolated from the tracheobronchial lymph nodes of 21 of 22 pigs, and from the corresponding lungs of 12 of 16 pigs which were studied bacteriologically.

Experiment III: BCG Infection Without Silicosis, Infection Controls

A control group of 24 healthy adult guinea pigs (tables 3 and 4) was infected intracutaneously on the same day and with the same suspension of BCG used

in the two previous experiments. The course of the tissue reaction at the site of the intracutaneous infection was no different from that observed in the animals exposed to quartz dust. In general, however, the tuberculin skin tests in this control group were more positive and ischemia with ulceration was a common feature. Furthermore, the response to the second tuberculin test did not show the degree of waning observed in the silicotic animals of the previous two experiments.

In contrast to the silicotic animals, these infection control nonsilicotic pigs exhibited some striking differences. First, none of the animals died from natural causes; all were killed. Second, and more striking, is the fact that spreading tuberculosis was not found in the lungs of any of the animals even as long as 608 days after the intracutaneous infection (figures 15 and 16). The tracheobronchial lymph nodes were only moderately enlarged. In only 2 animals did the nodes exhibit microscopic evidence of tuberculosis, which was strictly localized and was discrete and regressive in type. Third, and equally striking, is the finding that, in spite of the predominantly negative histopathology, cultures positive for tubercle bacilli were obtained from the nodes of 8 of 19 animals studied bacteriologically. In one animal a positive culture was obtained from the tracheobronchial nodes 547 days after the intracutaneous localization of the bacilli. Cultures from the lungs, however, remained consistently negative.

Experiments IV, V, and VI: H37Ra Infection Occurring Simultaneously with Developing Silicosis, Occurring Superimposed upon an Established Silicosis, and Occurring Without Silicosis

The guinea pigs in this group of experiments (tables 5, 6, 7, and 8) were observed at intervals as long as 608 days after the intracutaneous infection. A positive skin reaction to Old Tuberculin was elicited in all animals tested at 78 days, and again by the survivors tested at 295 days after infection. As was noted for the previous group of animals infected with BCG, the reactions to the second tuberculin test in this group of experiments employing the H37Ra organism were also less intense than to the first tuberculin test. Moreover, in general, the silicotic pigs were less sensitive than the nonsilicotic infection control animals. During the course of these experiments, 11 animals died from intercurrent nontuberculous pneumonia and one died from an unknown cause. The remaining 58 animals were killed at intervals. In every instance the lungs and tracheobronchial lymph nodes failed to reveal evidence of tuberculous infection (figures 17 to 22). Tubercle bacilli were not isolated from the lungs of any of the animals studied bacteriologically. However, as early as 63 days and as late as 608 days after infection, tubercle bacilli were cultured from the tracheobronchial lymph nodes of 11 of 65 animals. It is pertinent to note here that all of these animals with cultures positive for tubercle bacilli belonged to the groups (tables 5 and 6) which had been exposed to quartz dust and that 9 of the positive cultures were derived from animals which had received their intracutaneous infection after they had been exposed to quartz dust for 120 days.

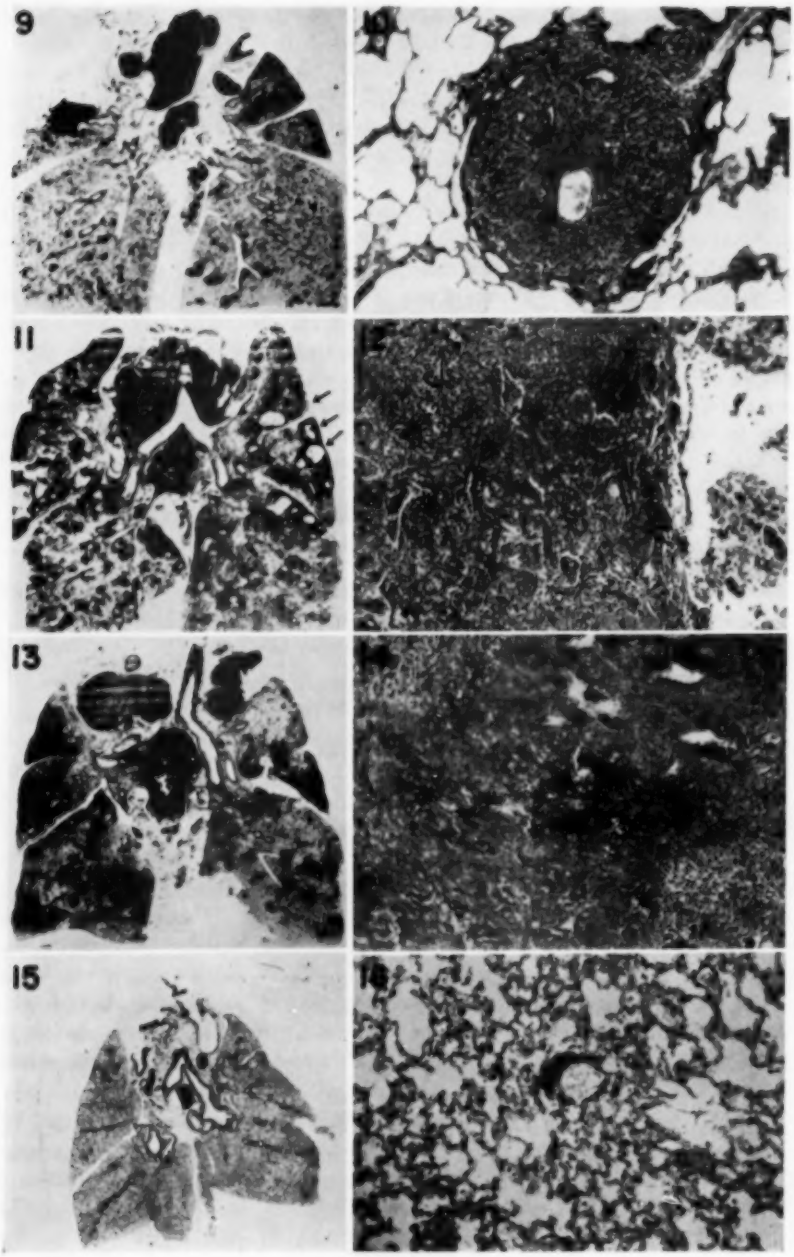

PLATE II

Experiments VII, VIII, and IX: M. Marinum Infection Occurring Simultaneously with Developing Silicosis, Occurring Superimposed upon an Established Silicosis, and Occurring Without Silicosis

Since the findings in this group of experiments were essentially negative, a detailed presentation and corresponding tables have been omitted from this report. Only a brief review of the results will be given. The first tuberculin skin test, at 78 days after the intracutaneous injection of the bacillary suspension, was positive in all animals. The reactions ranged from 1-plus to 2-plus in the silicotic pigs and 2-plus to 3-plus in the nonsilicotic infection control pigs. At 295 days the skin reactions of the silicotic pigs to the second tuberculin test were less intense, being either negative or only a 1-plus response. In the nonsilicotic infection control pigs, however, the skin reactions remained predominantly 2-plus but occasionally were 3-plus. The infection at the intracutaneous

PLATE II

FIG. 9. *Experiment II.* Guinea Pig Number 14. Inhalation of quartz dust, then intracutaneous BCG (Aronson) followed by continued inhalation of quartz dust. Killed at 426 days. The gross lung reveals scattered small lesions which have coalesced, especially in both upper lobes. The tracheobronchial lymph nodes are enlarged. Histologic evidence of tuberculosis was not discovered in this animal although tubercle bacilli were isolated from the nodes. (1.5 ×)

FIG. 10. Photomicrograph of the reaction to inhaled quartz dust about a vascular trunk in the lung (figure 9). The reaction is predominantly cellular but a thin zone of hyalinized tissue borders the upper margin of the blood vessel. (200 ×)

FIG. 11. *Experiment II.* Guinea Pig Number 21. Inhalation of quartz dust, then intracutaneous BCG (Aronson) followed by continued inhalation of quartz dust. Died at 363 days. Advanced coalescing tuberculosilicosis with liquefaction necrosis and scattered small cavities in the lung. The lymph nodes are enlarged with liquefying tuberculosilicosis. (1.5 ×)

FIG. 12. Photomicrograph of tuberculosilicotic tissue about a cavity present in the lung (figure 11). The cavity seen on the right contains purulent material. A thin membrane is detached from the tuberculosilicotic tissue to the left. In the latter, note the purulent necrotic material which separates the strands of fibrous tissue. (200 ×)

FIG. 13. *Experiment II.* Guinea Pig Number 25. Inhalation of quartz dust, then intracutaneous BCG (Aronson) followed by continued inhalation of quartz dust. Died at 475 days. Advanced confluent tuberculosilicosis. The tracheobronchial lymph nodes are enlarged and the carinal node exhibits a small cavity due to loss of liquefied necrotic material. (1.5 ×)

FIG. 14. Photomicrograph of necrotic area in a silicotic node complicated by tuberculous infection present in animal (figure 13). The necrosis involves primarily the hyalinized fibrous tissue which is visualized as thick interlacing bands in the left lower quadrant of the picture. (200 ×)

FIG. 15. *Experiment III.* Guinea Pig Number 78. Intracutaneous BCG (Aronson) infection control animal killed at 608 days. The lungs and lymph nodes are essentially normal. (1.5 ×)

FIG. 16. Photomicrograph of pulmonary tissue from animal (figure 15). The alveolar spaces are normally irregular and disproportional in size. The alveolar walls are thin. At the center is visualized a thin-walled vascular trunk partially surrounded by a small aggregate of lymphoid tissue usually present in the lung of a guinea pig. There is no histologic evidence of tuberculosis. (200 ×)

TABLE 3

EXPERIMENT III

INTRACUTANEOUS BCG (ARONSON): INFECTION CONTROLS

GUINEA PIG NUMBER	DATE OF INFECTION	TUBERCULIN SKIN TEST		MODE OF DEATH	TIME AFTER INFECTION	TUBERCULOSIS		ORGANISMS ISOLATED	
						Lungs	Tracheobronchial Lymph Nodes	Lungs	Tracheobronchial Lymph Nodes
		January 24, 1951	August 29, 1951		*days*				
56	November 7, 1950	—	—	K	62	0	NS	*	P
57	November 7, 1950	—	—	K	62	0	0	*	0
58	November 7, 1950	3+	—	K	127	0	0	*	P
59	November 7, 1950	4+	—	K	127	0	0	*	P
60	November 7, 1950	4+	—	K	181	0	0	0	P
61	November 7, 1950	4+	—	K	181	0	0	0	0
62	November 7, 1950	4+	—	K	241	0	0	0	P
63	November 7, 1950	4+	—	K	241	0	0	0	P
64	November 7, 1950	4+	2+	K	304	0	0	0	0
65	November 7, 1950	3+	2+	K	304	0	0	0	0
66	November 7, 1950	4+	2+	K	364	0	0	0	0
67	November 7, 1950	4+	4+	K	364	0	0	0	0
68	November 7, 1950	3+	3+	K	426	0	0	0	P
69	November 7, 1950	4+	4+	K	426	0	0	0	0
70	November 7, 1950	4+	4+	K	489	0	0	0	0
71	November 7, 1950	4+	3+	K	489	0	0	0	0
72	November 7, 1950	3+	2+	K	547	0	0	0	0
73	November 7, 1950	4+	3+	K	547	0	0	0	P
75	November 7, 1950	3+	3+	K	608	0	0	0	0
76	November 7, 1950	3+	3+	K	608	0	0	*	*
77	November 7, 1950	3+	3+	K	608	0	0	*	*
78	November 7, 1950	4+	4+	K	608	0	0	*	*
79	November 7, 1950	4+	4+	K	608	0	0	*	*
80	November 7, 1950	4+	4+	K	608	0	NS	*	*

Key: *see* Table 1.

site of inoculation gave rise initially to a small focal area of induration which persisted for approximately two months and which ultimately disappeared, leaving occasionally a barely visible scar. Except for being slightly enlarged, the draining inguinal and iliac nodes exhibited no significant abnormality. In all animals there was no gross or microscopic evidence of tuberculosis, and all cultures of the lungs and of the tracheobronchial lymph nodes remained consistently negative for *M. tuberculosis*.

Experiment X: Silicosis Without Infection

The guinea pigs exposed to quartz dust in *Experiments IV*, *V*, *VII*, and *VIII* would serve as the silicotic control animals since their lungs and tracheobronchial lymph nodes (figures 17 to 20) remained free of detectable histopathologic evidence of complicating tuberculosis. In order to obviate debate, it seems es-

TABLE 4

SUMMARY OF DATA: BCG (ARONSON) INFECTION

EXPERIMENT	EXPERIMENT I INTRACUTANEOUS BCG AND QUARTZ INHALATION SIMULTANEOUSLY	EXPERIMENT II QUARTZ INHALATION FOR 120 DAYS, THEN INTRACUTANEOUS BCG WITH QUARTZ INHALATION CONTINUED	EXPERIMENT III INTRACUTANEOUS BCG INFECTION CONTROLS
Duration of observations after infection	608 days	475 days	608 days
Number of guinea pigs observed	25	25	24
Number of guinea pigs killed	20	13	24
With tuberculosis of the lungs and of the tracheobronchial lymph nodes	1	5	0
With tuberculosis of the tracheobronchial lymph nodes only	7	4	2
Without tuberculosis	12	4	22
Number of guinea pigs died	5	12	0
With tuberculosis of the lungs and tracheobronchial lymph nodes	3	9	0
With tuberculosis of the tracheobronchial lymph nodes only	2	2	0
Without tuberculosis	0	1	0
Number of guinea pigs with organisms isolated from the lungs and/or the tracheobronchial lymph nodes	12	21	8

sential to present briefly the picture of simple silicosis as seen in the lungs and tracheobronchial lymph nodes of guinea pigs exposed to quartz dust but unchallenged by infection with tubercle bacilli. Observations on this question were derived from a series of healthy adult pigs which lived in the same room and which were exposed to the same atmospheric concentration of quartz dust as were the animals in the previous experiments. Furthermore, the observations are based also upon past studies of silicosis as it developed in a large number of guinea pigs whose quartz dust exposure was comparable in every respect to the experiments currently reported. These studies have been described as early as 1932 in numerous publications from this laboratory, especially by Gardner (4).

The silicotic reaction in the lungs and in the tracheobronchial lymph nodes of the guinea pigs exposed to quartz dust by inhalation consists initially of large mononuclear phagocytes which engulf the dust particles impinged upon the walls of the alveolar spaces. Subsequently, the phagocytes carry the particles into the alveolar

TABLE 5

EXPERIMENT IV

INTRACUTANEOUS H37Ra AND QUARTZ INHALATION SIMULTANEOUSLY

GUINEA PIG NUMBER	DATE OF INFECTION	TUBERCULIN SKIN TEST		MODE OF DEATH	TIME AFTER INFECTION	TUBERCULOSIS		ORGANISMS ISOLATED	
						Lungs	Tracheobronchial Lymph Nodes	Lungs	Tracheobronchial Lymph Nodes
		January 24, 1951	August 29, 1951		*days*				
111	November 7, 1950	—	—	K	63	0	0	*	*
112	November 7, 1950	—	—	K	63	0	0	*	P
113	November 7, 1950	3+	—	DPn	123	0	0	*	*
114	November 7, 1950	3+	—	K	127	0	0	*	0
117	November 7, 1950	3+	—	K	127	0	0	*	0
115	November 7, 1950	3+	—	K	181	0	0	0	0
116	November 7, 1950	2+	—	K	181	0	0	0	P
118	November 7, 1950	4+	—	K	241	0	0	0	0
135	November 7, 1950	3+	—	K	241	0	0	0	0
119	November 7, 1950	3+	2+	K	304	0	0	0	0
120	November 7, 1950	3+	2+	K	304	0	0	0	0
121	November 7, 1950	3+	2+	K	364	0	0	0	0
122	November 7, 1950	2+	2+	K	364	0	0	0	0
123	November 7, 1950	3+	2+	K	426	0	0	0	0
124	November 7, 1950	2+	2+	K	426	0	0	0	0
125	November 7, 1950	2+	2+	K	489	0	0	0	0
126	November 7, 1950	3+	2+	K	489	0	0	0	0
134	November 7, 1950	3+	2+	DPn	541	0	0	0	0
127	November 7, 1950	2+	2+	K	547	0	0	0	0
128	November 7, 1950	4+	2+	K	547	0	0	0	0
132	November 7, 1950	3+	2+	D	601	0	0	0	0
131	November 7, 1950	4+	2+	K	608	0	0	0	0
133	November 7, 1950	3+	2+	K	608	0	0	0	0

Key: *see* Table 1.

walls and into the intrapulmonary lymph channels, especially those about the vascular trunks. By way of those channels the particles are drained to the lymphoid aggregates within the lungs and to the nodes about the bronchi and trachea. With prolonged exposure and as the quartz particles concentrate in the tissue, small focal lesions consisting of closely packed, large mononuclear cells and an occasional multinucleated giant cell appear. Similar focal lesions make their appearance also throughout the parenchymatous portion of the lung. As the lesions enlarge and mature, the local connective tissue elements proliferate with the formation of collagen and fibrosis. Eventually, the lesions become dominated by hyalinized fibrous tissue which microscopically appears as compact interlacing bands forming masses of relatively acellular tissue. Usually, the masses are surrounded by zones of chronic inflammatory cells which infiltrate into the adjacent tissue. In general, the lesions are irregular in shape because they extend along the course of the alveolar walls and because they merge with one another to replace large areas of the pulmonary parenchyma (figures 23 and 24).

TABLE 6

EXPERIMENT V

QUARTZ INHALATION FOR 120 DAYS, THEN INTRACUTANEOUS H37Ra WITH QUARTZ INHALATION CONTINUED

GUINEA PIG NUMBER	DATE OF INFECTION	TUBERCULIN SKIN TEST		MODE OF DEATH	TIME AFTER INFECTION	TUBERCULOSIS		ORGANISMS ISOLATED	
						Lungs	Tracheobronchial Lymph Nodes	Lungs	Tracheobronchial Lymph Nodes
		January 24, 1951	August 29, 1951		*days*				
87	November 7, 1950	—	—	DPn	44	0	0	*	*
88	November 7, 1950	—	—	K	63	0	0	*	0
105	November 7, 1950	—	—	K	63	0	0	*	P
85	November 7, 1950	—	—	DPn	77	0	0	*	*
83	November 7, 1950	2+	—	K	127	0	0	*	0
84	November 7, 1950	2+	—	K	127	0	0	*	0
81	November 7, 1950	2+	—	K	181	0	0	0	P
86	November 7, 1950	2+	—	K	181	0	0	0	P
89	November 7, 1950	3+	—	DPn	191	0	0	*	0
90	November 7, 1950	3+	—	DPn	191	0	0	*	0
95	November 7, 1950	2+	—	DPn	191	0	0	*	0
96	November 7, 1950	2+	—	DPn	191	0	0	*	0
97	November 7, 1950	4+	—	DPn	191	0	0	*	0
82	November 7, 1950	2+	—	K	241	0	0	0	0
98	November 7, 1950	3+	—	K	241	0	0	0	P
91	November 7, 1950	3+	2+	K	364	0	0	0	P
92	November 7, 1950	3+	2+	K	364	0	0	0	P
94	November 7, 1950	3+	2+	DPn	414	0	0	0	P
99	November 7, 1950	3+	2+	K	426	0	0	0	0
100	November 7, 1950	3+	2+	K	426	0	0	0	0
101	November 7, 1950	3+	2+	K	489	0	0	0	P
102	November 7, 1950	3+	2+	K	489	0	0	0	0
103	November 7, 1950	2+	2+	K	547	0	0	0	0
104	November 7, 1950	2+	2+	K	547	0	0	0	0
93	November 7, 1950	2+	2+	K	608	0	0	0	P

Key: *see* Table 1.

It is well known that the early pulmonary lesion produced by inhaled quartz dust bears some similarity to the early tubercle (7) and that identification of the one lesion as a complication of the other may be difficult. Differentiation is especially troublesome in instances of acute reaction in the lung resulting from the deposition of large amounts of crystalline free silica. Under the conditions of the experiments of this report, however, such acute tissue reaction to the inhaled quartz dust did not occur. The tuberculous component of the tuberculosilicotic process in the various animals of those experiments was detected histopathologically by a combination of a number of the following findings: polymorphonuclear leukocytic infiltration, focal presence of compact epithelioid cells, swelling and poor definition of the hyalinized bands of fibrous tissue, evidence of

TABLE 7

EXPERIMENT VI

INTRACUTANEOUS H37Ra: INFECTION CONTROLS

GUINEA PIG NUMBER	DATE OF INFECTION	TUBERCULIN SKIN TEST		MODE OF DEATH	TIME AFTER INFECTION	TUBERCULOSIS		ORGANISMS ISOLATED	
						Lungs	Tracheobronchial Lymph Nodes	Lungs	Tracheobronchial Lymph Nodes
		January 24, 1951	August 29, 1951		*days*				
136	November 7, 1950	—	—	K	63	0	0	*	0
137	November 7, 1950	—	—	K	63	0	0	*	0
138	November 7, 1950	4+	—	K	127	0	0	*	0
139	November 7, 1950	4+	—	K	127	0	0	*	0
140	November 7, 1950	4+	—	K	181	0	0	0	0
141	November 7, 1950	4+	—	K	181	0	0	0	0
142	November 7, 1950	4+	—	K	241	0	0	0	0
143	November 7, 1950	3+	—	K	241	0	0	0	0
144	November 7, 1950	3+	4+	K	304	0	0	0	0
145	November 7, 1950	4+	2+	K	304	0	0	0	0
149	November 7, 1950	3+	4+	DPn	358	0	0	*	*
146	November 7, 1950	4+	2+	K	364	0	0	0	0
147	November 7, 1950	3+	2+	K	364	0	0	0	0
155	November 7, 1950	4+	2+	K	408	0	0	0	0
148	November 7, 1950	4+	2+	K	426	0	0	0	0
150	November 7, 1950	3+	2+	K	426	0	0	0	0
151	November 7, 1950	4+	2+	K	489	0	0	0	0
152	November 7, 1950	4+	4+	K	489	0	0	0	0
153	November 7, 1950	—	—	K	547	0	0	0	0
154	November 7, 1950	—	—	K	547	0	0	0	0
156	November 7, 1950	—	—	K	608	0	0	0	0
157	November 7, 1950	—	—	K	608	0	0	0	0

Key: *see* Table 1.

caseating and liquefying necrosis, occurrence of cavitary disease, and presence of acid-fast bacilli. These findings were conspicuous by their absence in the simple silicotic lesions uncomplicated by tuberculous infection.

Experiment XI: Virulence of Organism Isolated from the Lungs and the Tracheobronchial Lymph Nodes

Throughout the course of *Experiments I* to *IX*, inclusive, portions of the lungs and of the lymph nodes of various animals were cultured for tubercle bacilli (table 9). Observations in this regard will be reported more fully in a separate publication. Here it suffices to record that the organisms isolated on culture were tested for virulence by injecting 10 mg. of the culture growth subcutaneously into the right groin of each of 4 healthy adult guinea pigs.

A total of 164 subinoculated guinea pigs were used for the virulence tests of cultured bacilli isolated from animals infected with the BCG organism. The pigs killed at 30 and 60 days showed subcutaneous abscess at the site of inoculation with slight enlargement and moderate induration of the homolateral in-

TABLE 8

SUMMARY OF DATA: H37RA INFECTION

EXPERIMENT	EXPERIMENT IV INTRACUTANEOUS H37Ra AND QUARTZ INHALATION SIMULTANEOUSLY	EXPERIMENT V QUARTZ INHALATION FOR 120 DAYS, THEN INTRACUTANEOUS H37Ra WITH QUARTZ INHALATION CONTINUED	EXPERIMENT VI INTRACUTANEOUS H37Ra INFECTION CONTROLS
Duration of observations after infection	608 days	608 days	608 days
Number of guinea pigs observed	23	25	22
Number of guinea pigs killed	20	17	21
With tuberculosis of the lungs and of the tracheobronchial lymph nodes	0	0	0
With tuberculosis of the tracheobronchial lymph nodes only	0	0	0
Without tuberculosis	20	17	21
Number of guinea pigs died	3	8	1
With tuberculosis of the lungs and of the tracheobronchial lymph nodes	0	0	0
With tuberculosis of the tracheobronchial lymph nodes only	0	0	0
Without tuberculosis	3	8	1
Number of guinea pigs with organisms isolated from the lungs and/or the tracheobronchial lymph nodes	2	9	0

guinal and iliac nodes. At 120 days the abscess was usually healed and the nodes were only slightly enlarged. It is important to note that, in spite of the large challenging infection, none of the 164 animals exhibited evidence of spread of disease to the lungs, to the tracheobronchial nodes, or to other organs.

With reference to the animals infected with the H37Ra strain of organism, the virulence test of isolated bacilli involved 44 healthy adult guinea pigs. Here, too, the subcutaneous site of inoculation developed an abscess which ultimately regressed and healed. The draining inguinal and iliac lymph nodes became temporarily enlarged. The inflammatory reaction remained localized, and all of the animals failed to exhibit any evidence of organ tuberculosis.

DISCUSSION

The results of the studies of the present report and the studies reported previously leave little doubt that attenuated tubercle bacilli and avirulent tubercle bacilli, injected intracutaneously into the left flank of guinea pigs, may dis-

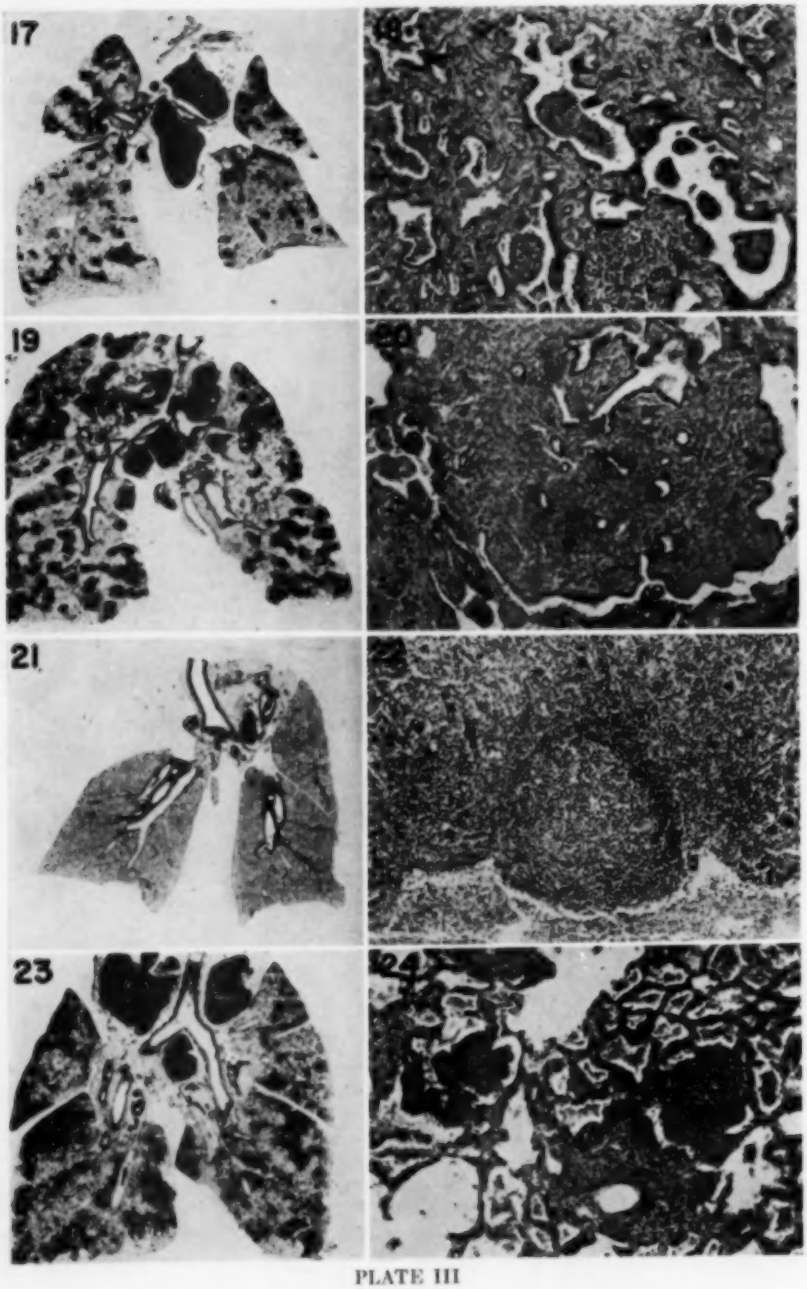

PLATE III

seminate from the site of inoculation in some, if not all, of the guinea pigs and may become localized in the lungs and tracheobronchial lymph nodes.

The studies also demonstrate that, when the attenuated BCG strains of *M. tuberculosis* (Aronson strain 873 and Rosenthal strain 826L) are used, the localized bacilli may remain viable for as long as 608 days and 543 days, respectively. In these circumstances, the bacilli may produce progressive and often fatal tuberculosis in tissue damaged by the presence of inhaled quartz dust. Moreover, the bacilli may be recovered from the tuberculosilicotic tissue and show no increase in virulence or change of the inherent attenuation when subinoculated into healthy adult guinea pigs.

PLATE III

FIG. 17. *Experiment IV*. Guinea Pig Number 128. Intracutaneous H37Ra and quartz inhalation simultaneously. Killed at 547 days. The photograph reveals irregular silicotic lesions scattered throughout the lung. The tracheobronchial lymph nodes are enlarged. There is no gross evidence of tuberculosis. (1.5 ×)

FIG. 18. Photomicrograph of hyalinizing fibrous tissue and cellular infiltration comprising the silicotic reaction which thickens the alveolar walls and obliterates the alveolar spaces in the lung shown in figure 17. Some of the spaces are lined by a thin layer of cuboidal epithelium and contain small masses of exudate, a common feature of chronic fibrosing disease in the lung of the guinea pig. There is no microscopic evidence of definite tuberculosis. (200 ×)

FIG. 19. *Experiment V*. Guinea Pig Number 103. Inhalation of quartz dust, then intracutaneous H37Ra followed by continued inhalation of quartz dust. Killed at 547 days. The gross section shows the lung studded with irregular coalesced lesions of silicosis. The tracheobronchial lymph nodes are enlarged. The lesions are larger than those in figure 17 because the animal of figure 19 was exposed for 120 days to quartz dust prior to the infection. (1.5 ×)

FIG. 20. Photomicrograph shows the character of the silicotic reaction present in the lung (figure 19). The reaction is dominated by hyalinizing fibrous tissue. Note the thickening of the alveolar walls and the compression with obliteration of the alveolar spaces. There is no microscopic evidence of definite tuberculosis. (200 ×)

FIG. 21. *Experiment VI*. Guinea Pig Number 153. Intracutaneous H37Ra infection control. Killed at 547 days. The lung and the tracheobronchial lymph nodes are essentially normal. Close inspection of the photograph may reveal scattered small pinpoint dark foci which represent the intrapulmonary aggregates of lymphoid tissue commonly found in the lung of healthy guinea pigs. Tuberculosis was not detected. The left upper lobe was removed for culture. (1.5 ×)

FIG. 22. Photomicrograph of germinal center at periphery of tracheobronchial lymph node shown in figure 21. The center is pale and slightly larger than normal. Tuberculosis was not detected. (200 ×)

FIG. 23. *Experiment X*. Guinea Pig Number 250. Inhaled quartz dust control. Poor condition; killed at 550 days. The picture shows the silicotic reaction scattered throughout the lung and enlarging the tracheobronchial lymph nodes. There is also an unusual confluence of the reaction due to a complicating nontuberculous pneumonia. There is no evidence of tuberculous infection. (1.5 ×)

FIG. 24. Photomicrograph of the lung seen in figure 23. The pulmonary reaction to the inhaled quartz dust has formed irregular lesions which follow the course of the alveolar walls. Note the distortion of the alveolar spaces, the cellular fibrotic tissue about the vascular trunk at the lower right, the relatively acellular hyalinized fibrous lesion to the left of center, the phagocytic cells and some exudate in many of the alveolar spaces. (150 ×)

TABLE 9

SUMMARY OF DATA ON CULTURES OF TISSUES FROM GUINEA PIGS INFECTED WITH BCG (ARONSON) AND WITH H37Ra ORGANISMS

EXPERIMENTS	NUMBER OF ANIMALS CULTURED	NUMBER OF ANIMALS		NUMBER OF ANIMALS WITH POSITIVE CULTURES			ORGANISMS ISOLATED AFTER INFECTION
		with Negative Culture	with Postive Culture	in Both Lungs and Tracheobronchial Lymph Nodes	in Lungs Only	in Tracheobronchial Lymph Nodes Only	
							days
Experiment I							
Intracutaneous BCG infection and quartz dust inhalation simultaneously	25	13	12	5	0	7	581
Experiment II							
Quartz inhalation for 120 days, then intracutaneous BCG with quartz inhalation continued	22	1	21	12	0	9	475
Experiment III							
Intracutaneous BCG-infection controls	19	11	8	0	0	8	547
Experiment IV							
Intracutaneous H37Ra infection and quartz dust inhalation simultaneously	21	19	2	0	0	2	181
Experiment V							
Quartz inhalation for 120 days, then intracutaneous H37Ra with quartz inhalation continued	23	14	9	0	0	9	608
Experiment VI							
Intracutaneous H37Ra-infection controls	21	21	0	0	0	0	0

The present studies also reveal that, when the avirulent H37Ra strain is used, the localized bacilli may remain viable in the tracheobronchial lymph nodes for as long as 608 days after intracutaneous infection. In these circumstances, the bacilli are incapable of producing progressive tuberculosis in tissue damaged by quartz dust and may be recovered from the tuberculosilicotic tissue and show no virulence when subinoculated into healthy adult guinea pigs.

With respect to the *M. marinum* strain of tubercle bacilli, which is pathogenic for cold-blooded animals, the studies show that inhaled quartz dust neither alters the pathogenicity of that organism nor influences the native tissue immunity in warm-blooded animals such as the guinea pig.

From the experimental results it is obvious that, for the silicotic guinea pig at least, two strains of BCG cannot be considered as avirulent, or as virus fixé, and incapable of producing progressive and fatal tuberculosis. Thus, in the light of these findings the two BCG strains, i.e., Aronson strain 873 and Rosenthal strain 826L, must be considered as *attenuated* in contrast to the *truly avirulent* strain H37Ra and to the *nonpathogenic* strain *M. marinum*.

It appears that the progressive tuberculosis which arises in the silicotic guinea pigs from infection with BCG is due to a factor of increased tissue susceptibility rather than to an acquired virulence of the attenuated bacilli per se.

In this connection a number of points for discussion arise. One point pertains to the use of various terms such as *virulent*, *attenuated*, *avirulent*, *stable*, *virus fixé* for characterizing a particular strain of tubercle bacillus and its capacity to produce tuberculosis. It seems timely to suggest that such terms be used, not only in conjunction with the specific name or number of the strain, but also with reference to the species of host employed and the physiologic state of tissue involved. It is common knowledge that different animal species vary remarkably in their tissue response to similar infection with the same strain of tubercle bacillus (8). Furthermore, there are many influences, some quite obvious, others subtle, and still others unknown, which act upon tissue in one way or another and alter the course of a tuberculous infection in the same species of animal. The action of quartz dust has been established as one such adverse influence.

The second point for discussion relates to increased tissue susceptibility as the factor in the progression of the tuberculosis produced by BCG observed in this series of experiments. Increased tissue susceptibility connotes a loss of tissue resistance followed by propagation of the infecting bacilli and, consequently, a progressive tuberculosis. Apparently, this factor can operate without an accompanying change in the inherent attenuation of the infecting tubercle bacilli; in this case, BCG. An alternative explanation is that the attenuated bacilli acquire an inherent virulence which is temporary in the silicotic tissue and which disappears in the healthy tissue of the nonsilicotic guinea pig subinoculated with the organisms isolated from that tissue. Acceptance of that alternative implies that, for the silicotic guinea pig at least, the BCG strains used in these experiments are not stable or virus fixé and are capable of producing progressive tuberculosis. In contrast, the results of these experiments show that the H37Ra strain retains its avirulent character even though challenged by tissue containing quartz dust. The H37Ra strain is considered, therefore, as being truly stable or a virus fixé under the conditions of the present experiments.

The third point for discussion concerns the mechanism by which crystalline free silica in the form of quartz dust enhances tissue susceptibility to infection by the attenuated tubercle bacilli (BCG). As yet, the responsible mechanism has not been discovered either as it pertains to these experiments, to the previous experiments (1), or to the series of experiments which yielded similar results but which were conducted with the attenuated R_1 strain of tubercle bacillus (9–12). It has been reported (13) that small amounts of colloidal silica when added to artificial culture medium will enhance the growth of tubercle bacilli. It has not

been possible in this laboratory, however, to demonstrate that silica, either in the colloidal state or in the free crystalline state, has such an effect. There is experimental evidence which supports the view that finely divided crystalline free silica embedded in tissue favors the growth of tubercle bacilli which localize in that tissue (11, 12, 14, 15). Available evidence suggests that the silica particle does not accomplish that effect by a direct action upon the bacillus. It appears possible that the particle after prolonged residence within a tissue may neutralize an inhibitory growth substance (16) present within that tissue or may stimulate the production of a substance inimical to local tissue immunity, thus allowing the bacilli to propagate and to produce progressive tuberculosis. Unfortunately, there is as yet no convincing evidence to substantiate those possibilities.

Progress in understanding the manner in which crystalline free silica adversely influences a tuberculous infection would be greatly facilitated if knowledge were available of the particular mechanism whereby silica exerts its toxic action upon tissue. Various theories have been proposed (17–30). Some of them are still being studied, but none has been accepted as a complete explanation of all observed phenomena. Thus far, the most satisfying theories involve either one or both of two seemingly complex physiochemical actions with the production of a substance which is toxic to the cell. One theory is predicated upon the production of a colloidal silica derived from the quartz particle. The other theory is based upon the denaturization of cellular protein adsorbed on the surface of the silica particles. It may be possible that the silica in solution or the denaturized and toxic protein alter the biochemical structure of the cell, thus creating a medium which enhances the susceptibility of the cell to infection by the tubercle bacillus. It is obvious, however, that the mechansim of such enhancement will remain obscure until there is a clear understanding of the manner in which silica, per se, injures the tissue cell.

SUMMARY

The experimental investigations substantiate previous studies concerning the BCG strain Rosenthal 826L and demonstrate the following:

Tubercle bacilli of the attenuated BCG strain Aronson 873 and of the avirulent human strain H37Ra may disseminate from an intracutaneous site of inoculation. The BCG organisms may seed the lungs and the tracheobronchial lymph nodes of normal and of silicotic guinea pigs. The H37Ra organisms may seed the tracheobronchial lymph nodes of silicotic pigs, but those organisms were not isolated from the nodes of the normal pigs or from the lungs of the normal or of the silicotic pigs. In contrast, bacilli of the nonpathogenic *M. marinum* strain remained localized at the intracutaneous site of inoculation and were not isolated from the lungs or nodes of any of the animals.

Tubercle bacilli of the attenuated BCG strain Aronson 873 may produce progressive and often fatal tuberculosis as a complication of the silicotic process in the lungs and tracheobronchial lymph nodes of guinea pigs with prolonged exposure by inhalation to crystalline free silica in the form of quartz dust. Tu-

bercle bacilli of the avirulent H37Ra strain and of the nonpathogenic *M. marinum* strain fail to accomplish such effects.

Tubercle bacilli isolated from the lungs and from the tracheobronchial lymph nodes of both the silicotic and the nonsilicotic guinea pigs retained the cultural characteristics of the organism (the attenuated BCG strain Aronson 873 and the avirulent strain H37Ra) originally injected intracutaneously into those pigs; and those bacilli exhibited no enhancement of virulence when subinoculated subcutaneously in large doses (10 mg.) into healthy guinea pigs.

The mechanism whereby crystalline free silica in the form of quartz dust deposited in tissue accomplishes its adverse effect upon a complicating tuberculous infection in silicotic guinea pigs appears to be a function of lowered tissue immunity rather than of direct action of the silica upon the infecting tubercle bacillus.

It must be emphasized that the observations noted in the present report were derived from experimental investigations with animals and that the findings apply only to the influence of inhaled quartz dust upon a tuberculous infection in guinea pigs.

SUMARIO

La Vacunación BCG en la Silicosis

Las investigaciones experimentales comprueban estudios anteriores acerca de la cepa BCG, la Rosenthal 826L, y demuestran lo siguiente:

Pueden diseminarse desde un sitio intracutáneo de inoculación bacilos tuberculosos de la cepa BCG atenuada, la Aronson 873, y de la cepa humana avirulenta, H37Ra. Los microbios BCG pueden sembrarse en el pulmón y en los ganglios linfáticos traqueobronquiales de cobayos normales y silicóticos. Los bacilos H37Ra pueden sembrar los ganglios linfáticos traqueobronquiales de cobayos silicóticos, pero no fueron aislados de los ganglios de los cobayos normales ni de los pulmones de los cobayos normales o los silicóticos. En contraposición, los bacilos de la cepa anapatógena *M. murinum* permanecieron localizados en el sitio intracutáneo de inoculación y no fueron aislados ni de los pulmones ni los ganglios de ninguno de los animales.

Bacilos tuberculosos de la cepa BCG atenuada, Aronson 873, pueden producir tuberculosis evolutiva y a menudo letal como complicación del proceso silicótico de los pulmones y los ganglios linfáticos traqueobronquiales de los cobayos con exposición prolongada por inhalación a la sílice libre cristalina en forma de polvo de cuarzo. Los bacilos tuberculosos de la cepa H37Ra avirulenta y de la cepa anapatógena *M. murinum* no provocan esos efectos.

Los bacilos tuberculosos aislados de los pulmones y los ganglios traqueobronquiales de los cobayos tanto silicóticos como no silicóticos retenían las características culturales del microbio (la cepa BCG atenuada, Aronson 873, y la cepa avirulenta, H37Ra) inyectado al principio intracutáneamente en dichos animales, y esos bacilos no manifestaron aumento de virulencia al ser subinoculados subcutáneamente a dosis grandes (10 mg.) en cobayos sanos.

El mecanismo por el cual la sílice libre cristalina en forma de polvo de cuarzo produce su efecto adverso sobre una infección tuberculosa complicante en cobayos silicóticos parece ser una función de hipoinmunidad de los tejidos más bien que acción directa de la sílice sobre los bacilos tuberculosos infectantes.

Debe recalcarse que las observaciones anotadas en este trabajo se derivan de investigaciones experimentales en animales y que los hallazgos rezan únicamente con el influjo del polvo de cuarzo inhalado sobre una infección tuberculosa en los cobayos.

RESUME

Vaccination par le BCG dans la silicose

Les investigations expérimentales confirment les études antérieures concernant la souche BCG et Rosenthal 826L, et démontrent ce qui suit:

Le bacille tuberculeux de la souche atténuée BCG, Aronson 873, et de la souche humaine avirulente, H37Ra, peut essaimer à partir du lieu d'inoculation intra-cutané. Les organismes BCG peuvent inoculer le poumon et les ganglions lymphatiques trachéo-bronchiques des cobayes normaux ou atteints de silicose. Les organismes H37Ra peuvent inoculer les ganglions trachéo-bronchiques des cobayes silicosés, mais ces bacilles n'ont pas été isolés à partir des ganglions de cobayes normaux ou des poumons du cobaye silicosé. Au contraire, les bacilles de la souche *M. marinum* non pathogène restaient localisés au foyer d'inoculation intra-cutané et ils n'ont été isolés dans les poumons ou les ganglions chez aucun des animaux.

Les bacilles tuberculeux de la souche atténuée BCG et de la souche Aronson 873, peuvent produire une tuberculose évolutive et souvent mortelle comme complication du processus de silicose dans les poumons et les ganglions lymphatiques trachéo-bronchiques des cobayes soumis à l'exposition prolongée, par inhalation de silice libre cristallisée sous la forme de poussière de quartz. Les bacilles tuberculeux de la souche H37Ra a avirulente et la souche *M. marinum* non pathogène ne produisent pas ces effets.

Les bacilles tuberculeux isolés des poumons et des ganglions trachéo-bronchiques des cobayes atteints de silicose ou chez ceux indemnes de silicose conservaient les caractéristiques culturales du germe (souche BCG atténuée, Aronson 873, et souche a virulente H37Ra) initialement injecté au cobaye par voie intra-cutanée; ces bacilles ne présentaient aucun accroissement de leur virulence quand ils étaient inoculés à dose élevée (10 mg) par voie intra-cutanée chez des cobayes en bonne santé.

Le mécanisme par lequel la silice libre cristallisée, sous la forme de poussière de quartz déposée dans les tissus, accompli son action nocive sur une infection tuberculeuse superposée chez le cobaye atteint de silicose, semble être le résultat de l'abaissement de l'immunité tissulaire plutôt qu'une action directe de la silice sur le bacille tuberculeux infectant.

Il y a lieu d'insister sur le fait que les observations figurant dans cet article ont été obtenues au cours d'investigations expérimentales sur les animaux, et que les résultats présentés concernent exclusivement l'influence de la poussière de quartz inhalée sur l'infection tuberculeuse du cobaye.

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THE EFFECT OF HORMONAL PREPARATIONS ON THE SURVIVAL OF MICE INJECTED INTRAVENOUSLY WITH VIRULENT, ATTENUATED, AND AVIRULENT MYCOBACTERIA[1,2]

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INTRODUCTION

The deleterious effect of cortisone on the course of experimentally induced tuberculous infection in several species of laboratory animals is well recognized (1). In mice, cortisone has been shown to produce exacerbation of relatively stable tuberculous infection and to enhance the more acute infection produced by the injection of relatively large numbers of tubercle bacilli (2). In addition, cortisone can eliminate the protection against tuberculous infection afforded mice by vaccination with BCG (3).

Large doses of cortisone and corticotropin (ACTH) can so lower the natural resistance of normal rats that fatal spontaneous systemic infections may occur with organisms of their normal body flora (4). Normal mice given large doses of cortisone also may show a bacteremia consisting of organisms generally regarded as normal inhabitants of their gastrointestinal tract (5). Furthermore, when death supervenes, as it usually does in those animals in which treatment with cortisone is prolonged, grayish nodular lesions are frequently seen in one or more of the viscera. These nodules, upon bacteriologic examination, may yield corynebacteria (5).

Recently, Selye has reported that the action of cortisone in reducing the resistance of normal rats to spontaneous infection was completely reversed by the administration of somatotropic hormone (STH) (6, 7). Also, STH was reported by Lemonde, Panisset, Dobija, and Selye (8) to exert a pronounced favorable effect in cortisone-treated rats infected intraperitoneally with the virulent H37Rv strain of *Mycobacterium tuberculosis* var. *hominis*. In addition, according to these same writers, "Preliminary experiments on mice infected with the same human type tuberculosis bacilli suggest that, in a normally tuberculous-sensitive species, STH exerts a protective effect even if the animal is not specifically sensitized by cortisone."

In view of the relation of some of the above reported findings to the problem of the nature of resistance to tuberculous infection, the experiments to be reported herein were carried out.

METHODS

Hormone preparations: Cortisone was given as the microcrystalline suspension of cortisone acetate (Merck Co., Inc.). *Corticotropin* (ACTH), *somatotropic hormone* (STH), and *thyrotropin* (TSH) were all furnished through the courtesy of Dr. Sanford L. Steelman,

[1] From the Department of Bacteriology, Northwestern University Medical School, Chicago, Illinois.

[2] This work was aided, in part, by a research grant from Parke, Davis & Company, Detroit, Michigan.

Fundamental Research Department, The Armour Laboratories, Chicago. Corticotropin was from lot # 212-103 and assayed 200 per cent of the LA1A standard. The somatotropic hormone employed was from lot # R-264158 and assayed 75 per cent of the 22KR2 standard. Thyrotropin was from lot # 317-52 and assayed 100 per cent of the 2R3 standard. The desired amounts of the respective preparations contained in a total volume of 0.2 ml. of physiologic saline were administered once daily by subcutaneous injection. In the first experiment (table 1), when two preparations were given simultaneously to the same animal, both substances were contained in the same 0.2 ml. volume. In subsequent experiments, however, the preparations were given separately.

The organisms employed were the virulent strain, H37Rv, of *M. tuberculosis* var. *hominis*; the attenuated bovine strain, BCG; the attenuated human strain, R1Rv[3]; and the avirulent human variant, H37Ra[3]. The method for the maintenance of the virulence of the H37Rv strain and the methods employed for preparation and standardization of suspensions for injection and the manner in which the standardized acute tuberculous infection was produced in mice have all been previously described in detail (9). Briefly, however, albino mice of the Strong A strain, weighing between 18 and 22 gm., were inoculated intravenously with 1.0 mg. of a mechanically prepared suspension of the virulent H37Rv strain. The effect of the infecting dose or the effect of the hormonal preparation on the infection so produced in mice was measured by recording the time of death of each mouse and plotting the cumulative percentage of deaths against time of death in days on logarithmic probability paper. From the graph the median survival time may be obtained. The statistical analysis with the calculation of the 95 per cent confidence limits was done by the method recommended by Litchfield (10). At the time of death, each mouse was autopsied and the viscera were examined. Mice which were injected with BCG, R1Rv, and H37Ra received 1.0 mg. of a suspension of the respective organism by the intravenous route. The suspension of BCG was taken from a BCG vaccine (lot 978P)[4] and was injected into the mice on the same day it was prepared.

RESULTS

The data showing the effect of the hormonal preparations given both singly and in combination on the tuberculous infection produced by the intravenous inoculation of the H37Rv strain are recorded in table 1. Whether given individually or in combination, the only preparation which produced any significant effect upon the course of the infection was cortisone. This agent reduced the median survival time of the mice to a value almost one-half that of the controls.

The data obtained upon employing a larger dose of the somatotropic hormone are shown in table 2. Again, there is no evidence that the somatotropic hormone ameliorated in any way the action of cortisone.

In view of the fact that Selye (6) had reported that the somatotropic hormone would counteract the lethal effect of cortisone in normal animals, an experiment also was conducted in which a group of normal mice was treated with cortisone, and a group of normal mice was treated with both cortisone and somatotropic hormone. These data are also included in table 2 and it is evident that, while the dosage of cortisone employed resulted in death of normal mice, the concomitant administration of somatotropic hormone did not significantly increase the survival of mice so treated.

[3] Obtained from Mr. William Steenken, Jr., Trudeau Laboratories, Trudeau, New York.

[4] Furnished through the courtesy of Dr. Sol R. Rosenthal, Tice Clinic, University of Illinois, Chicago, Illinois.

TABLE 1

THE EFFECT OF HORMONAL PREPARATIONS GIVEN SINGLY AND IN COMBINATION ON THE SURVIVAL OF MICE INFECTED WITH MYCOBACTERIUM TUBERCULOSIS VAR. HOMINIS (H37Rv)

NUMBER OF MICE	DRUG ADMINISTERED	DOSE PER DAY IN MICROGRAMS	MEDIAN SURVIVAL TIME IN DAYS (ST_m) (95 PER CENT CONFIDENCE LIMITS)
20	Corticotropin	50	15.0 (14–16)
20	Cortisone	500	7.5 (7.0–8.0)
20	Somatotropic hormone	200	11.5 (11–12)
20	Thyrotropin	500	13.0 (12–14)
20	Corticotropin	50	12.0 (11–13)
	Somatotropic hormone	200	
20	Corticotropin	50	12.0 (11.5–12.5)
	Thyrotropin	500	
20	Cortisone	500	7.2 (7.0–7.4)
	Somatotropic hormone	200	
20	Cortisone	500	7.4 (7.2–7.6)
	Thyrotropin	500	
20	None	None	13.0 (12–14)

TABLE 2

THE EFFECT OF CORTISONE AND SOMATOTROPIC HORMONE ON THE SURVIVAL OF NORMAL MICE AND ON THE SURVIVAL OF MICE INFECTED WITH M. TUBERCULOSIS VAR. HOMINIS (H37Rv)

NUMBER OF MICE	TYPE OF INFECTION	DRUG ADMINISTERED	DOSAGE IN MICROGRAMS PER DAY	MEDIAN SURVIVAL TIME IN DAYS (ST_m) (95 PER CENT CONFIDENCE LIMITS)
15	H37Rv	Cortisone	500	7.0 (6.5–7.5)
15	H37Rv	Cortisone	500	7.0 (6.5–7.5)
		Somatotropic hormone	500	
15	None	Cortisone	500	16.0 (11–24)
15	None	Cortisone	500	16.5 (13–20)
		Somatotropic hormone	500	
10	H37Rv	None	None	12.0 (11–13)

The reaction of cortisone-treated mice to inoculation with the avirulent strain, H37Ra, and to inoculation with the attenuated strains, BCG and R1Rv, is shown in table 3. The median survival times of the groups of mice inoculated with these three organisms and treated with cortisone did not differ significantly from the median survival times of uninoculated animals when treated with cortisone. The noncortisone-treated control mice inoculated with these three strains, upon autopsy, after being sacrificed months after being injected, showed grossly no evidence of tuberculosis. These results indicate that mice, whose resistance to tuberculous infection with virulent strains apparently has been markedly lowered by the injection of cortisone, still are not susceptible to infection with these avirulent or attenuated variants of *M. tuberculosis*.

TABLE 3

EFFECT OF CORTISONE ON THE SURVIVAL OF MICE INJECTED WITH AVIRULENT AND ATTENUATED MYCOBACTERIA

NUMBER OF MICE	ORGANISM INJECTED INTRAVENOUSLY	DRUG ADMINISTERED	DOSAGE IN MICROGRAMS PER DAY	MEDIAN SURVIVAL TIME IN DAYS (ST_{50}) (95 PER CENT CONFIDENCE LIMITS)
20	H37Rv	None		14.5 (13–16)
20	H37Ra	None		* Sacrificed at 12 months
20	H37Ra	Cortisone	500	16.5 (14–19)
20	None	Cortisone	500	16.5 (13–20)
20	BCG	None		Sacrificed at 75 days
20	BCG	Cortisone	500	12.5 (11–14)
20	None	Cortisone	500	13.0 (11.5–14.5)
20	R1Rv	None		* Sacrificed at 55 days
20	R1Rv	Cortisone	500	16.0 (13–19)
20	None	Cortisone	500	14.5 (12–18)

* Four mice died before time of sacrifice of causes other than tuberculosis.

DISCUSSION

Under the conditions of the experiment, cortisone was the only one of four hormonal preparations which significantly affected the survival times of mice infected with large doses of the H37Rv strain of *Mycobacterium tuberculosis* var. *hominis*. The effect of cortisone treatment resulted in a reduction of the survival time by approximately one-half as compared with infected controls not treated with this drug. These results confirm those of other investigators (2). The present results failed to confirm, however, the report of Selye (6, 7) that somatotropic hormone reverses the lethal effect of cortisone and also failed to confirm the finding of Lemonde, Panisset, Dobija, and Selye (8) that experimental tuberculosis of rats and mice can be favorably influenced by the administration of somatotropic hormone. The reason for this discrepancy in results is not immediately apparent. The hormonal preparation employed in the present studies had been assayed and found to be fully potent. The dosage of cortisone, on a per kilogram basis, was approximately one-sixth the dosage employed by Selye (6) for the production of spontaneous infections in normal rats. Furthermore, the maximal dosage of somatotropic hormone employed in the present experiments, while approximately one-half the amount employed by Selye on a per kilogram basis, was nevertheless three to four times as much, relative to the amount of cortisone given, as that employed by Selye.

The short report by Lemonde, Panisset, Dobija, and Selye (10) on experimental tuberculosis in mice does not include sufficient data to permit any comparison. The objection might be raised that the tuberculous infection produced in the mice in the present experiment was so acute and overwhelming that even an appreciable degree of increased resistance afforded by somatotropic

hormone might be obscured. That such a possibility exists cannot be denied. However, the method is highly sensitive to changes in the state of resistance of mice to infection with virulent mycobacteria. This can be demonstrated by the increase in the median survival time produced by vaccination of mice with small amounts of BCG vaccine and other antigenic preparations (11). Any increase in resistance brought about by somatotropic hormone would be of low degree if it is not detectable by this method.

Of particular interest is the response of mice which received cortisone to the injection of the avirulent H37Ra strain and the attenuated BCG and R1Rv strains. In no case did the median survival time of these animals differ significantly from the median survival time of normal animals inoculated with cortisone. Furthermore, upon autopsy, none of the mice injected with either of these two strains showed any evidence of tuberculous infection. In fact, the organs could not be distinguished from those of the control normal mice which received cortisone. Their organs resembled the description of the viscera of normal mice treated with cortisone given by Berlin, Johnson, Hawk, and Lawrence (5).

It is apparent from these data that reduction by cortisone of the resistance of mice to a point where microorganisms from the intestinal or respiratory tract, usually considered to be nonpathogenic, can produce spontaneous infection and death, nevertheless, did not alter the susceptibility to infection of these mice to the avirulent H37Ra strain or to the attenuated BCG and R1Rv strains.

The reaction of cortisone-treated mice to infection with the virulent H37Rv strain indicates that this treatment interfered with some resistance mechanisms of the mouse, perhaps permitting the infection to progress virtually unchecked. Although all of the actions of cortisone which might affect resistance to infection cannot be defined, it has been demonstrated that, in mice, cortisone markedly reduces the phagocytic activity of the reticulo-endothelial system and apparently retards the macrophage response (12). Gell and Hinde (13), however, have furnished evidence which indicates that the action of cortisone is directed toward inhibiting the phagocytic motility rather than the phagocytic activity. This would seem to confirm the generally recognized importance of these cells for innate resistance to tuberculous infection. Conversely, however, the lack of pathogenicity of the avirulent and attenuated strains for cortisone-treated mice might suggest that the lack of ability of these strains normally to produce progressive disease may be less a reflection of their susceptibility to normal defense mechanisms than, perhaps, to an innate inability to continue multiplication in the *in vivo* nutritional environment.

On the other hand, the defense mechanisms affected by cortisone may not comprise all of those available to the host for the suppression of tuberculous infection. Those unaffected by cortisone might be adequate to cope with organisms possessing only a limited capacity to multiply *in vivo*.

Further data on the reaction of actively immunized, cortisone-treated mice to infection with virulent tubercle bacilli would be informative in this respect. Such experiments are being conducted and will be reported subsequently.

SUMMARY

Mice infected intravenously with 1.0 mg. of the virulent H37Rv strain of *Mycobacterium tuberculosis* var. *hominis* were treated with cortisone, corticotropin (ACTH), somatotropic hormone (STH), and thyroid-stimulating hormone (TSH). Cortisone treatment markedly reduced the median survival time. None of the other hormonal preparations significantly affected the survival of the tuberculous mice. Somatotropic hormone, when administered to cortisone-treated tuberculous mice, did not ameliorate to any degree the deleterious effects of the cortisone on the course of the infection. Furthermore, somatotropic hormone did not prevent or modify the spontaneous infection evoked in normal mice following the administration of cortisone.

Treatment of mice with cortisone did not increase the susceptibility of mice to infection with the attenuated BCG and R1Rv strains, nor to the avirulent H37Ra strain.

SUMARIO

El Efecto de las Preparaciones Hormónicas sobre la Sobrevivencia de los Ratones Injectados Intravenosamente con Micobacterias Virulentas, Atenuadas y Avirulentas

Ratones infectados intravenosamente con 1.0 mg. de la cepa virulenta H37Rv del *Mycobacterium tuberculosis* var. *hominis* fueron tratados con cortisona, corticotropina (ACTH), hormona somatotropa (HST) y hormona estimulante del tiroides (HST). La cortisonoterapia rebajó considerablemente el tiempo medio de sobrevivencia. Ninguna de las otras preparaciones hormónicas afectó significativamente la sobrevivencia de los ratones tuberculosos. La hormona somatotropa, al ser administrada a los ratones tuberculosos tratados con cortisona, no mejoró mayor cosa los efectos nocivos de la cortisona sobre la evolución de la infección. Además, tampoco impidió o modificó la infección espontánea producida en los ratones normales por la cortisona.

El tratamiento de los ratones con la cortisona no acrecentó la susceptibilidad de los mismos a la infección con las cepas atenuadas BCG y R1Rv, ni a la cepa avirulenta H37Ra.

RESUME

L'effet des préparations hormonales sur la survie des souris inoculées par voie veineuse avec des mycobactéries virulentes, avirulentes ou atténuées

Après inoculation intraveineuse de 1 mg. de la souche H37Rv virulente du *M. tuberculosis* var. *hominis*, des souris ont été traitées par la cortisone, la corticotrophine (ACTH), l'hormone somatotrope (STH) ou l'hormone thyréotrope (TSH). Le traitement par la cortisone a réduit notablement le temps de survie moyen. Aucune des autres préparations hormonales n'a modifié de façon appréciable la survie de la souris tuberculisée. L'hormone somatotrope, administrée à la souris traitée par la cortisone, n'a amélioré à aucun degré l'action nocive de la cortisone sur le cours de l'infection. De plus, l'hormone somatotrope n'a pas prévenu ou modifié l'infection spontanée se produisant chez une souris normale ayant été traitée par la cortisone.

Le traitement des souris par la cortisone n'a pas augmenté la sensibilité de l'animal à l'infection par les souches atténuées BCG et R1Rv ou par la souche H37Ra avirulente.

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HEMIN AS A GROWTH FACTOR FOR CERTAIN ISONIAZID-RESISTANT STRAINS OF MYCOBACTERIUM TUBERCULOSIS[1,2]

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INTRODUCTION

There is little doubt that, in general, the acquisition of the property of drug resistance by a microorganism is associated with an altered metabolism. In some instances, such an alteration may be reflected by the development of a "growth factor" requirement, as exhibited by the failure of the resistant variant to grow in a medium which was suitable for the growth of the parent strain. This has been demonstrated, for example, with *Escherichia coli* in two separate studies. Kohn and Harris (1) found that certain sulfanilamide-resistant strains became dependent on methionine, and Rosanoff and Sevag (2) have shown that some strains made resistant to streptomycin had acquired a need for a number of amino acids. As reported previously (3), a similar phenomenon has been observed in these laboratories in the case of certain types of tubercle bacilli resistant to isoniazid. The present publication is a description of the experiments and results which have demonstrated that the iron porphyrin *hemin* will function as a growth factor for these particular isoniazid-resistant strains.

It should be emphasized at the outset that this growth factor phenomenon is associated only with certain isoniazid-resistant variants, particularly those which appear after a single or initial exposure to rather high concentrations of isoniazid, similar to the "one-step" mutants described by Szybalski and Bryson (4). As shall be shown in a separate publication (5), isoniazid-resistant strains of tubercle bacilli may be obtained which can grow well in a synthetic medium, and, therefore, exhibit no growth-factor requirement.

METHODS

Only liquid media were employed in these experiments, and incubation was at 36° to 38°C. in an air incubator. Except for the basal medium, which was autoclaved, all other materials used as enrichment were rendered bacteria free by positive pressure Seitz filtration. Stock aqueous solutions of isoniazid were generally made up at a concentration of 1,000 γ per ml., autoclaved, and stored in the refrigerator until used. The parent culture for these studies was the H37Rv strain of *Mycobacterium tuberculosis* var. *hominis*, the growth of which, by the methods to be described, was completely inhibited after fourteen days' incubation in the basal medium by concentrations of 0.006 to 0.012 γ of isoniazid per ml. Henceforth, the symbol *H37Rv* will refer to the isoniazid-susceptible, non-factor-requiring parent strain; and *isoniazid-resistant FR* will designate those strains which were proved to be isoniazid resistant and factor requiring.

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[1] From the Research Laboratories of Parke, Davis & Company, Detroit 32, Michigan.

[2] Presented in part at the Seminar on *Bacteriology of Tuberculosis in Relation to Isonicotinic Acid Derivatives* at the annual meeting of the National Tuberculosis Association, Los Angeles, California, on May 21, 1953.

The *basal medium* used in these studies consisted of:

Asparagine	0.10 gm.
$Na_2HPO_4 \cdot 12H_2O$	0.63 gm.
KH_2PO_4	0.10 gm.
Na_3citrate$\cdot 2H_2O$	0.15 gm.
$MgSO_4 \cdot 7H_2O$	0.02 gm.
Glycerol	2.0 gm.
Distilled water	100.0 ml.

Prior to autoclaving, the pH of this medium was adjusted to 6.8 with concentrated HCl.

Preparation of Inocula

M. tuberculosis H37Rv: The H37Rv strain was received from Dr. G. P. Youmans of Northwestern University in October, 1951, and stock cultures have been maintained since then as pellicle growths on shallow layers of the basal medium. Pellicle-to-pellicle transfers are made at fourteen-day intervals. Suspensions to be used as inocula were obtained by inoculation of tubes of Tween-albumin media (basal medium plus 0.02 per cent Tween 80[2] and 0.2 per cent Fraction V albumin, Armour) with portions of pellicle. After seven days' incubation, growth in the Tween-albumin medium was diluted in the basal medium and turbidimetrically standardized, using a Coleman model 7 photonephelometer (6), to contain approximately 100 million cells per ml. For each 5 ml. of test medium, 0.1 ml. of such a suspension was employed as the inoculum.

M. tuberculosis, isoniazid-resistant FR: Although details of the characteristics of the isoniazid-resistant FR strain will be published separately (5), a few pertinent points should be mentioned. After fourteen days' incubation in twofold serial dilutions of isoniazid in Tween-albumin medium, growth of the parent H37Rv strain was completely inhibited by 0.024 γ of isoniazid per ml. On further incubation, and between twenty-two and thirty days following the original inoculation, growth appeared almost simultaneously in all tubes containing up through 6 γ of isoniazid per ml. Such growth, when tested for isoniazid susceptibility in the basal medium plus 10 per cent bovine serum, proved to be isoniazid resistant as evidenced by prompt growth in isoniazid concentrations as high as 25 or 50 γ per ml. This applied to the growth which appeared in isoniazid concentrations of 0.05 γ per ml. or higher. However, only the strains appearing in isoniazid concentrations of approximately 1 γ per ml., or more, proved incapable of growth in the basal medium. Such strains were classed as factor-requiring or "FR" cultures. It was noted, furthermore, that cultures of this sort, although remaining isoniazid resistant, would appear to lose the need for a growth factor if they were subcultured three or four times at seven-day intervals through Tween-albumin medium *in the absence of isoniazid*. On the other hand, if they were maintained in Tween-albumin media with as little as 0.05 γ of isoniazid per ml., they appeared to be "stable" for at least ten weeks in so far as the factor-requirement characteristic was concerned. In general, therefore, FR strains to be used as inocula were maintained as stock cultures in Tween-albumin medium plus 1.0 γ of isoniazid per ml. The standardization of such inocula was the same as that previously described for its H37Rv strain.

Methods for Identification of the Growth Factor

Direct assay: The principle of this type of test is the same as that generally used in any microbiologic procedure for the characterization of a growth factor in that a deficient medium is employed with one or more substances added to it for growth response evaluation. In this case, a given substance to be assayed for growth factor properties was added to the basal medium ("deficient medium") according to a twofold dilution scheme, and

[2] Tween 80 is the proprietary name for the polyoxyethylene derivative of Sorbitan monooleate, obtainable from the Atlas Powder Co., Wilmington, Delaware.

was then inoculated with the FR type of culture. Such a series was inspected after seven and fourteen days' incubation for evidence of growth.

Resistance emergence: This method was employed to complement the "direct assay" procedure on the assumption that the FR type of variant would appear in high isoniazid concentrations on prolonged incubation *only in those media containing the growth factor*. The correctness of this assumption will be discussed later. In a given experiment, twofold serial dilutions of isoniazid with a maximum concentration of 100 γ per ml. were prepared in the basal medium and in the same medium enriched with a particular substance. The H37Rv strain was used as the inoculum, and incubation was carried out for at least seven weeks, with frequent inspection for the appearance of presumably isoniazid-resistant variants. The series in the basal medium served as a "negative" control, since in many such tests the appearance of "one-step" resistant variants has never been observed. If growth appeared after several weeks' incubation in the enriched medium series in isoniazid concentrations greater than 1.0 γ per ml., it was subcultured to Tween-albumin medium and then tested for the salient FR characteristics: isoniazid resistance, failure to grow in the basal medium, and good growth in a 10 per cent bovine serum medium. Invariably, these subcultures of delayed growth in isoniazid concentrations greater than 1 γ per ml. proved to be of the FR type. A given substance permitting this sort of "resistance emergence" was assumed to contain or to be the growth factor.

OBSERVATIONS

Listed in tables 1 and 2 are so-called "negative" materials, i.e, those which failed to support the growth of the FR variant by direct assay and those which did not permit the emergence or appearance of such variants in isoniazid concentrations in excess of 1.0γ per ml. Because of the inherent laboriousness of the "resistance emergence" procedure, fewer substances were tested by this method than by the "direct assay" procedure.

It will be noted that yeast extract, folic acid, nicotinamide, and pantothenate are included in the "negative" group although, in the previous publication (3), "positive" or variable growth factor properties were ascribed to these. There is good reason to believe that the earlier experiments with these substances were of questionable reliability due to the fact that "pure" FR-type strains were probably not employed and that the growth which occurred in these enrichments consisted in reality of a small residuum of the original isoniazid-susceptible H37Rv population (5).

As shown in table 3, FR strains appeared in the basal medium (in isoniazid concentrations of at least 1.0γ per ml.) enriched with bovine serum or Fraction V albumin, with the time of appearance of FR growth roughly related to the concentration of enrichment. Also, there appears to be a minimum amount of serum or Fraction V albumin which will accomplish this, namely approximately 0.1 per cent of serum and 0.5 per cent of Fraction V albumin.

The test results which were directly responsible for the characterization of hemin as a growth factor are presented in table 4. In this case, it was demonstrated that the factor was probably present in erythrocytes and, subsequently, hemoglobin, as determined by the "resistance emergence" method. The erythrocyte lysate was prepared by suspension of 1 per cent of bovine erythrocytes (calculated from packed volume) in distilled water, with lysis accomplished by hypotonicity combined with alternate freezing and thawing. The results with

TABLE 1

MATERIALS INACTIVE AS GROWTH FACTOR FOR ISONIAZID-RESISTANT STRAIN OF TUBERCLE BACILLI

("Direct Assay" Method)

MATERIAL TESTED	RANGE OF CONCENTRATIONS TESTED (γ PER ML. BASAL MEDIUM)	
	Maximum	Minimum
Ascorbic acid	100	0.02
p-Aminobenzoic acid	10,000	20.0
p-Hydroxybenzoic acid	10,000	0.00002
Orotic acid	1.0	0.00002
Proline	1,000	0.2
Glycine	1,000	0.2
Glutamate (Na)	1,000	0.2
Succinate (NH_4)	1,000	0.2
β-Alanine	10	0.00002
Nicotinic acid	10,000	0.00002
Isonicotinic acid	10,000	0.00002
Nicotinamide	10,000	0.00002
Pantothenate (Ca)	1,000	0.000002
Riboflavin	1,000	0.000002
Folic acid	1,000	0.000002
Pyridoxal (HCl)	1,000	0.000002
Pyridoxine (HCl)	1,000	0.000002
Thiamine (HCl)	1,000	0.000002
m-Inositol	100	0.2
Biotin	10	0.02
Pantetheine	2	0.000004
Diphosphopyridine nucleotide (DPN, Coenzyme I)	10.0	0.02
Triphosphopyridine nucleotide (TPN, Coenzyme II)	10.0	0.02
Vitamin Mixture:		
Nicotinamide	50	0.1
Pantothenate (Ca)	50	0.1
Thiamine (HCl)	1.0	0.002
Riboflavin	50	0.1
Ascorbic acid	100	0.2
Biotin	1.0	0.002
	PER CENT CONCENTRATIONS	
	Maximum	Minimum
Liver extract*	5.0	0.00001
Yeast extract†	0.5	0.00001
Serum ultrafiltrate‡	10.0	0.02
Crystalline albumin§	5.0	0.01
Bovine Serum Proteins‖		
Proantithrombin	1.0	0.002
Thrombin	1.0	0.002
Fibrinolysin	1.0	0.002
Fibrinogen	1.0	0.002
Prothrombin	1.0	0.002
Antifibrinolysin	1.0	0.002
Accelerator globulin	1.0	0.002

* Parke, Davis & Co.

† Difco autolyzed fresh yeast.

‡ Microbiological Associates (from ox serum).

§ Armour.

‖ Kindly furnished by Mr. E. C. Loomis, Parke, Davis & Co.

TABLE 2

MATERIALS INACTIVE BY THE "RESISTANCE EMERGENCE" METHOD

MATERIAL	CONCENTRATIONS
Yeast extract*	0.5, 0.25, 0.125, 0.06, and 0.03%
Liver extract†	0.1, 0.05, 0.01, and 0.005%
Crystalline albumin‡	0.2%
Tween 80	0.02%
Folic acid	1.0 γ per ml.
Nicotinamide	1.0 γ per ml.
Pantothenate (Ca)	1.0 γ per ml.
Riboflavin	10.0, 5.0, 1.0, 0.5, & 0.1 γ per ml.
Pyridoxal (HCl)	100.0 and 10.0 γ per ml.
Pyridoxine (HCl)	100.0 and 10.0 γ per ml.
Biotin	10.0 γ per ml.
Succinate (NH_4)	0.1%
Glutamate (Na)	0.1%
Glycine	0.1%
Arginine	0.1%
Histidine	0.1%

* Difco autolyzed fresh yeast.
† Parke, Davis & Co.
‡ Armour.

TABLE 3

POSITIVE RESULTS OBTAINED WITH BOVINE SERUM AND FRACTION V ALBUMIN ("RESISTANCE EMERGENCE" METHOD)

DAYS	PER CENT ENRICHMENT CONCENTRATION AND CORRESPONDING M.I.C.* ISONIAZID, γ PER ML.					
	Bovine Serum					
	10.0	5.0	1.0	0.5	0.1	0
5	0.024	0.049	0.024	0.024	0.006	0.006
11	0.098	0.049	0.024	0.024	0.024	0.006
18	25.0	12.5	0.39	0.024	0.024	0.012
25	50.0	25.0	12.5	3.13	0.39	0.024
38	50.0	50.0	25.0	12.5	1.56	0.024
	Fraction V Albumin					
	5.0		1.0	0.5	0.1	
5	0.049		0.024	0.006	0.006	
11	0.098		0.049	0.012	0.006	
18	25.0		1.56	0.049	0.024	
25	100.0		25.0	6.25	0.39	
38	100.0		25.0	6.25	0.78	

* Minimal inhibitory concentration.
† Armour.

TABLE 4

POSITIVE RESULTS OBTAINED WITH BOVINE ERYTHROCYTE LYSATE AND HEMOGLOBIN ("RESISTANCE EMERGENCE" METHOD)

DAYS	M.I.C.* ISONIAZID, γ PER ML. ERYTHROCYTE LYSATE (TESTED ONLY AT 1.0 PER CENT)			
6	0.024			
14	0.78			
21	100.0			
28	>100.0			
	HEMOGLOBIN† (CONCENTRATIONS IN γ PER ML.)			
	50.0	25.0	12.5	6.25
7	0.024	0.024	0.006	0.003
14	50.0	0.024	0.006	0.003
21	50.0	0.024	0.006	0.003
28	50.0	0.049	0.012	0.006
35	50.0	1.56	0.024	0.006

* Minimal inhibitory concentration.
† Armour, bovine.

TABLE 5

MATERIALS FROM BOVINE BLOOD WHICH SUPPORTED GROWTH OF ISONIAZID-RESISTANT (FACTOR-REQUIRING) M. TUBERCULOSIS

("Direct Assay" Method)

Serum (per cent)	*10.0*	*5.0*	*2.5*	*1.25*	*0.625*	*0.313*
Growth*	++++	++++	++++	+++	++	0
V Albumin† (per cent)	*5.0*	*2.5*	*1.25*	*0.625*	*0.313*	
Growth	++++	++++	++	0	0	
Hemoglobin‡ (γ per ml.)	*500*	*250*	*125*	*62.5*	*31.3*	*15.6*
Growth	++	+++	++++	++++	+	0
Globin† (γ per ml.)	*100*	*50*	*25*	*12.5*		
Growth	±	±	0	0		
Hemin‡ (γ per ml.)	*10.0*	*5.0*	*2.5*	*1.25*	*0.625*	*0.313*
Growth	++++	++++	++++	++++	±	0

* Degree of growth after fourteen days, approximated.
† Armour.
‡ Eastman, 97+ per cent pure.

hemoglobin indicated that a minimum concentration of approximately 25γ per ml. was necessary to promote the appearance of FR variants. It seemed most appropriate, therefore, to test hemin and globin by the "direct assay" method for their ability to support the growth of the FR strain. The results of such tests, including the evaluation of bovine serum and Fraction V albumin, are given in table 5.

On the basis of the evidence summarized in table 5, it was concluded that hemin was the growth factor.

DISCUSSION

As the minimum amount of hemin, under the conditions described, which will support the growth of the isoniazid-resistant FR variants is approximately 1γ per ml. of basal medium, this amount, at least, should be present in 0.6 per cent of bovine serum, 1 per cent of Fraction V albumin, and 62γ of hemoglobin per ml. of basal medium. Evidence has been obtained that such a concentration of hemin may be present in Fraction V albumin. Spectrophotometric analysis of this albumin has revealed the presence of absorption bands characteristic of hemin or some closely related porphyrin. This analysis, in addition, indicated that this porphyrin is present at a concentration of approximately 0.01 per cent in Fraction V albumin. It is also likely, then, that the growth factor activity of serum may be due largely to its content of a hemin-albumin complex, and secondarily to some hemoglobin or free hemin. The activity of hemoglobin is doubtless due to its hemin component, and the slight activity of globin may be ascribed to contaminating traces of hemin.

A series of experiments, the details of which will not be presented here, indicated that the growth factor was non-dialyzable from bovine serum or Fraction V albumin and that it was stable to heating at 60°C. for thirty minutes. It was not extractable from bovine serum by any of the following solvents: heptane, benzene, diethyl ether, ethylene dichloride, chloroform, *n*-butanol, or ethyl acetate. Also, preliminary work with bovine serum indicated that no clear-cut separation of this factor was achieved by paper-strip electrophoresis. These observations are consistent with the expected behavior of hemin as it exists in Fraction V albumin and bovine serum.

It is apparent, from the data given in tables 1 through 5, that there was good agreement between the two methods ("direct assay" and "resistance-emergence") both qualitatively and quantitatively. Materials which did not act as a growth factor for the FR variant by "direct assay" were also inactive by the "resistance emergence" method. On the other hand, substances such as serum, Fraction V albumin, and hemoglobin were "positive" in both procedures. Of even greater significance is the fact that the minimum effective concentrations of these three substances are almost identical in both the "direct assay" and "resistance emergence" types of tests.

One may conclude, therefore, that *"one-step" resistance of tubercle bacilli to isoniazid will occur in vitro only if hemin is present in a sufficient amount.* Conversely, such resistance will not be apparent if hemin is absent or rendered unavailable. This generalization, from "H37Rv" to "tubercle bacilli," is supported by the fact that results similar to those described here for the H37Rv strain were obtained with a number of human strains of recent clinical origin, as well as with bovine varieties (5).

The role of hemin as a microbial growth factor is certainly not unusual. It has long been known that exogenous hemin is necessary for the growth of some *Hemophilus* species (7) and for members of the genus *Trypanosoma* (8). Also, a strain of yeast has been described which requires protoporphyrin IX (9). With the aforementioned microorganisms, hemin, according to the criteria

described by Snell (10), would be classified as a growth factor. Furthermore, the most likely explanation for such a hemin requirement is that these organisms have lost the ability to synthesize this porphyrin. In the case of the FR type of variant described here, the situation appears to be analogous, namely that hemin is needed as a growth factor and that these types of isoniazid-resistant tubercle bacilli are unable to synthesize hemin.

It appears that, as the apparent loss of a hemin-synthesizing mechanism is associated with resistance to isoniazid, this represents one means by which tubercle bacilli may by-pass isoniazid inhibition. The extension of this hypothesis would indicate that isoniazid interferes with the metabolism of hemin by isoniazid-susceptible tubercle bacilli. This is supported by the observation made here, and to be published (11), that hemin antagonizes and reverses isoniazid inhibition of the growth of tubercle bacilli. Since, as Granick has suggested (12), porphyrin synthesis may have a common or identical metabolic pathway in a variety of organisms and since isoniazid is active as a growth inhibitor almost specifically for tubercle bacilli, it must be concluded that some aspects of porphyrin metabolism of the tubercle bacillus are unique and that these are particularly vulnerable to attack by isoniazid.

SUMMARY

Evidence has been presented that hemin functions as a growth factor for certain isoniazid-resistant variants obtained from the H37Rv strain of *M. tuberculosis*. It was also concluded that "one-step" resistance to relatively high concentrations of isoniazid would occur *in vitro* only in an environment which supplied sufficient hemin. The hypothesis is presented that the loss of a hemin-synthesizing mechanism renders tubercle bacilli resistant to inhibition of growth by isoniazid, and that it is the porphyrin metabolism of tubercle bacilli which is peculiarly vulnerable to inhibition by isoniazid.

SUMARIO

La Hemina como Factor del Crecimiento para Ciertas Cepas Isoniacidorresistentes del Mycobacterium Tuberculosis

Preséntanse pruebas de que la hemina funciona como factor del crecimiento para ciertas variantes isoniacidorresistentes obtenidas de la cepa H37Rv del *M. tuberculosis*. Dedúcese además que la resistencia "en un tiempo" a concentraciones relativamente altas de isoniacida sólo ocurrirían *in vitro* en un ambiente que suministrara suficiente hemina. Ofrécese la hipótesis de que la pérdida de un mecanismo sintetizador de la hemina insensibiliza a la bacilos tuberculosos a la inhibición del crecimiento por la isoniacida, y que el metabolismo de la porfirina en los bacilos tuberculosos es el que es en particular vulnerable a la inhibición por la isoniacida.

RESUME

L'hémine comme facteur de croissance pour certaines souches isoniazide-résistantes du Mycobacterium tuberculosis

Présentation de données montrant que l'hémine agit comme facteur de croissance pour certaines variantes isoniazide-résistantes obtenues à partir de la souche H37Rv du *M. tuberculosis*. L'auteur conclut que la résistance de première génération ("one-step")

concentrations relativement élevées succédant à l'exposition à l'isoniazide en ne se produiraient *in vivo* que dans un milieu fournissant suffisamment d'hémine. L'auteur formule l'hypothèse que la perte du mécanisme de synthèse de l'hémine rend le bacille tuberculeux insensible à l'inhibition de la croissance par l'isoniazide et que c'est le métabolisme porphyrinique des bacilles tuberculeux qui est spécialement vulnérable à l'inhibition par l'isoniazide.

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FACTORS AFFECTING THE INTRADERMAL TUBERCULIN REACTION ON THE GUINEA PIG[1]

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INTRODUCTION

A number of writers (1, 2, 3) have reported differences between Old Tuberculin (OT) and purified protein derivative (PPD) in the nature of the dose-response relationship shown by experiments on guinea pigs. Although similar results were obtained by the different investigators, in no instance was the number of animals sufficient for tests of the difference between the dose-response curves to approach statistical significance. Some of the experiments also had faults of design which tended to reduce their accuracy still further.

It can be shown that, when the 6-point design is used and the dose ratio is 4:1, the numbers of guinea pigs required to measure accurately a difference in slope between two materials of 5, 10, 20, and 30 per cent are 332, 83, 21, and 9, respectively. It therefore seemed advisable to plan an assay of OT against PPD on a larger scale than has previously been reported, and this paper is in part concerned with the results of such an experiment.

The results were incidentally of some physiologic interest and are interpreted as showing that the injection of tuberculin or the reaction to it initiates a central mechanism which tends to depress the size of the reaction. Two further experiments are reported which were planned to throw more light on this aspect of the tuberculin reaction.

The present work was also designed to discover whether a level of dosage existed within which one could obtain a valid estimate of the relative potencies of the two materials. The experiments demonstrated that the dose-response relationships of the two materials were different at all levels, and this difference would tend to vitiate statistical significance tests. The acceptance of Seibert's PPD-S as a separate standard in addition to the existing international standard Old Tuberculin recognizes possible differences; the experiments reported below strongly support this action.

The response of tuberculin on the guinea pig has for many years been assumed to be proportional to the logarithm of the dose (log dose), although this point appears to have been made explicitly only in 1949 by Wadley (*4a*). It has also been shown (*4b*) that the response to johnin on the cow is proportional to log dose and implied (*4a*) that a similar relationship holds for a number of tuberculins, presumably of heat-concentrated synthetic medium type. The only report in which PPD is discussed (5) mentions that there were indications from a number of experiments that the dose-response lines differed between tuberculins. So far as can be discovered, a close study of the PPD response has not

[1] From the Veterinary Laboratory, Ministry of Agriculture and Fisheries, Weybridge, England.

TABLE 1

DILUTIONS USED IN EACH ASSAY

The Same Dilutions Were Used on May 11 and August 1, 1951

ASSAY NUMBER	DILUTIONS OF THE STOCK SOLUTION OF PPD OR OT			STRENGTH OF THE OT SOLUTION EXPRESSED IN INTERNATIONAL UNITS PER ML.		
	a	*b*	*c*	*a*	*b*	*c*
1	1:8,000	1:2,000	1:500	12.5	50	200
2	1:1,600	1:400	1:100	62.5	250	1,000
3	1:800	1:200	1:50	125	500	2,000
4	1:160	1:40	1:10	625	2,500	10,000

previously been made which might be expected to be basic to the others, since PPD is 95 per cent tuberculoprotein. The present paper aims at making good this deficiency to some extent.

The first experiment described below was a large-scale assay, planned for the purpose of investigating the relative potencies over four different dose ranges of a sample of commercial Old Tuberculin and a human PPD tuberculin prepared at this laboratory. The original objects were to determine: (*1*) if, relative to PPD, OT had a greater relative potency at high than at low concentration; (*2*) if any radical alteration occurred in the slopes of the dose-response curves at different levels of dosage; and (*3*) whether the assay could be repeated after an interval on the same set of guinea pigs with similar results.

MATERIALS AND METHODS

A commercial sample of Old Tuberculin (OT) was assayed against a solution containing 2 mg. of Weybridge purified protein derivative (PPD) per ml. The schedule of dilutions used is given in table 1. Each dilution was made from the concentrate by the addition of sterile normal saline.

The 60 male albino guinea pigs used were kept in twelve cages of 4 per cage and four cages of 3 per cage. The cages were allocated to the four assays so that each assay could be carried out on three cages of 4 pigs and one cage of 3 pigs. The pigs were fed on a pelleted diet (6), known as Diet 18, supplemented with as much kale as they would eat. They were sensitized by the injection into the muscles of the right thigh of 0.5 ml. of a suspension in paraffin containing 2 mg. per ml. of a steam-killed human strain. Each guinea pig, therefore, received 1 mg. of dead bacteria.

The tuberculins were injected into the skin of the dorsolateral body surface on May 11, 1951. In statistical terminology, each guinea pig was regarded as a block and the six doses to be injected were assigned to six sites on each animal at random to set up the well-known randomized block design. The four assays were repeated on August 1, 1951; for this occasion the randomization process was carried out afresh, and the results of the two sets of assays can, therefore, be regarded as independent. The diameter of the erythematous reaction was measured twenty-four hours after the time of injection. These diameters provided the data which have been analyzed below.

RESULTS

Assay of Old Tuberculin Against Purified Protein Derivative

The relative potency of PPD and OT: As the original purpose of the experiment was to investigate the relationship between PPD and OT responses, the results

TABLE 2

VALIDITY TESTS ON THE COMBINED RESULTS OF EIGHT ASSAYS; REGRESSION OF DIAMETER OF RESPONSE ON LOG DOSE

	d.f.	MEAN SQUARE	F	
			Observed	Value at P = 0.01
Mean slope	1	4,325.67		
Slope differences	1	12.33	6.60	6.64
Mean curvature	1	1.56		
Curvature differences	1	14.48	7.75	6.64
Error	300	1.8685		

TABLE 3

SLOPES AND CURVATURES OF DOSE-RESPONSE LINES IN WHICH DIAMETER OF RESPONSE IS RELATED TO LOG DOSE

The Set of May 11, 1951, on 15 Guinea Pigs; the Set of August 1, 1951, on 12 Guinea Pigs

DATE	ASSAY NUMBER	SLOPES		DIFFERENCE PPD − OT	CURVATURES		COMBINED CURVATURE	DIFFERENCE IN CURVATURE PPD − OT
		PPD	OT		PPD	OT		
May 11, 1951	1	102	98	4	−12	6	−6	−18
	2	94	105	−11	−10	5	−5	−15
	3	79	88	−9	−9	−10	−19	1
	4	86	116	−30	−8	16	8	−24
Total		361	407	−46	−39	17	−22	−56
August 1, 1951	1	69	78	−9	−7	8	1	−15
	2	70	75	−5	−16	1	−15	−17
	3	71	76	−5	−15	−8	−23	−7
	4	76	84	−8	−14	28	14	−42
Total		286	313	−27	−52	29	−23	−81
Grand total		647	720	−73	−91	46	−45	−137
Values for grand total significant at $P = 0.05$		56			70	70	97	97

will first be treated as a single assay. The investigation of some further points of interest will be dealt with in a later section.

Tests of validity (table 2) showed that no valid estimate of relative potency could be obtained from the combined results of these assays because the dose-response curves for the two materials were not parallel, the variance of slope differences being 6.6 times error variance.

Mean dose-response lines are shown in figure 1. When the slopes and curvatures of the dose-response lines are examined in detail, the numerical results (table 3) show that the main reason for the significant variances of table 2 was

negative curvature of the PPD lines. This curvature did not consistently appear in the OT lines, which, on the whole, adequately fitted the linear hypothesis tested.

Inspection of a series of 40 routine 6-point assays revealed a general tendency for the mean diameters of PPD responses to negative curvature when plotted against log dose. It must therefore be concluded that this dose-response relationship, although a useful first approximation, is inexact and must tend, on the average, to introduce a slight bias into the estimates of relative potency even of small-scale assays.

Investigation of the PPD data showed that, when mean area (represented by the square of the diameter) was plotted against log dose, the dose-response curves were all straightened (figure 2). Since the original (diameter) dose-response relationship fitted the OT data adequately, the new metameter cannot be expected to improve this fit. It was, in fact, found that, with area as the response metameter, the mean of the OT dose-response lines curved to a significant degree (table 4). There is thus no pair of metameters which will set up the conditions for a valid assay of OT in terms of PPD, i.e., parallel straight dose-response lines.

An estimate of relative potency with restricted implications can be obtained from the data by taking only the responses to the two lower doses of each tuberculin (where the lines are parallel). This estimate is used in the calculations

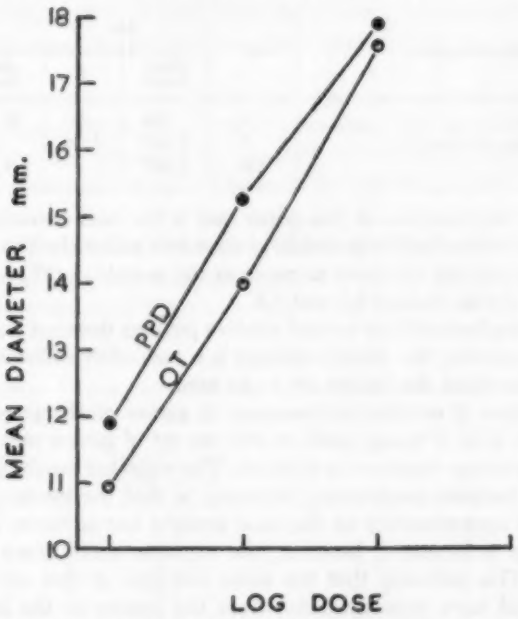

FIG. 1. Relative potency of PPD and OT: mean dose-response lines.

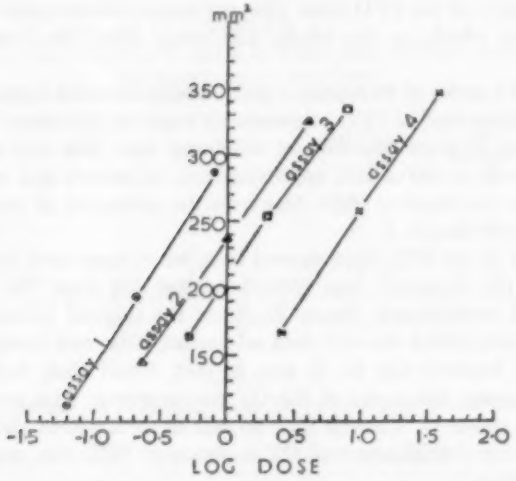

FIG. 2. Relative potency of PPD and OT: effect of level of dosage on average response.

TABLE 4

SIGNIFICANCE TESTS APPLIED TO THE REGRESSIONS OF AREA ON LOG DOSE

The 5 Per Cent Point of F Is 3.9

SOURCE OF VARIATION	d.f.	PPD		OT	
		Mean Square	F	Mean Square	F
Mean curvature	1	304	<1	23,017	15.41
Curvature X date of assay	1	1,597	1.58	222	<1
Error	104	1,007		1,494	

presented in a later section of this paper and is the best estimate available of the relative amounts of active principle in these two materials. The PPD solution is estimated to contain 1.8 times as much as the sample of OT, with 5 per cent fiducial limits of this ratio at 1.5 and 2.3.

It must be emphasized that a valid relative potency does not exist; the device adopted for obtaining the above estimate is a makeshift sufficiently sound for the purpose for which the figures are to be used.

The interactions of neighboring responses: A glance at the graphs in figure 2 shows that the level of dosage used on any one set of guinea pigs has a marked effect on the average response in that set. The expected result, in the absence of interaction between neighboring responses, is that the results of all four assays should be approximately on the same straight line or curve. Each time the level of dosage is increased, however, the response curve drops below its expected level. This indicates that the mean response of that set of animals is less than would have been predicted from the results at the lowest level of dosage. From the graphs (figure 2), the mean area of response due to an injection

of 2.5 γ of PPD (log 2.5 = 0.4) would be 296, 270, or 167 mm.^{2} according to whether it was given under the conditions of assay Nos. 2, 3, or 4. This degree of divergence clearly needs no statistical tests to establish its significance.

As all responses on the same animal are affected, it is reasonable to postulate that the effect is mediated through some central mechanism. The size of the depressing effect should, therefore, be a function of all the injections because all injected sites are able to contribute to the total effect. The physiologic aspects of this mechanism are referred to in the discussion, in relation to the results of two further experiments. The statistical problem is to find a simple equation capable of expressing the findings of the present experiment and likely to bear some relation to physiologic theory.

For the statistical purpose, the area of the response (y) was treated as the "dependent" variate and log dose at the site of injection y (designated x_1) as one "independent" variate in a bivariate regression analysis. The second independent variate (x_2) was a function of w, the total weight of tuberculin injected into the animal. Of the various functions which were tried, a good fit was obtained when $(fx_2) = \log^2 10w$, the regression equation being $y^2 = 146x_1 - 26x_2 + 333$. The coefficients of both x_1 and x_2 were highly significant, being 63 times and 36 times their respective standard errors.

The numerical value of w can be estimated from the present experiment only with some degree of approximation, because the exact amount of tuberculoprotein in the OT is unknown. The estimate of relative potency given in the first section under "Results" was used, with the known quantities of PPD injected, to calculate the total weight of tuberculin injected. The results are shown in table 5.

$\text{Log}^2\ w$ is an unsuitable function to use when the value of w is less than 3, because of the peculiarities introduced by squaring the logarithms of numbers lying between 0 and 2. The arbitrary factor 10 was therefore introduced to avoid this difficulty. Columns 3 and 4 of table 5 demonstrate the effect of this procedure.

Influence of Multiple Infection on Average Response

A further experiment was designed as a direct test of the hypothesis that the diameter of an intradermal tuberculin reaction would be influenced by the total

TABLE 5

TOTAL TUBERCULIN INJECTED PER ANIMAL IN EACH ASSAY

Stated in Terms of γ of PPD

ASSAY NUMBER	ESTIMATED TOTAL PPD INJECTED PER GUINEA PIG (w)	$x_2 = \log^2 10w$	$\log^2 w$
	γ		
1	1.65	1.46	0.044
2	8.20	3.65	0.083
3	16.5	4.88	1.46
4	82.0	8.47	3.65

amount of tuberculin received by the guinea pig at other sites as well as by the amount of tuberculin injected directly into the site.

Fourteen cages of 4 male albino guinea pigs, sensitized with dead organisms, as in the first experiment, were allocated at random to the block numbers of a "balanced incomplete block" design (7). The design provides a balanced arrangement whereby eight treatments may be grouped in blocks containing four units only; pairs of blocks form complete replications. In this experiment the "block" was a cage of 4 guinea pigs, each of which received a different treatment.

A guinea pig received i intradermal injections of tuberculin and $(8 - i)$ intradermal injections of normal saline ($i = 1, 2 \ldots 8$) given twenty-six days after the sensitizing injection. The volume of all injections was 0.2 ml. Tuberculin injections contained 2 γ of PPD in 0.2 ml. of normal saline. Eight injection sites were chosen, four on each dorsolateral surface; when i was less than 8, a table of random numbers was used to decide which sites were to have tuberculin.

In the animal house, the pairs of cages were kept in two rows, arranged so that only one cage of each pair making up a replicate occurred in each row.

The design of the experiment was such that there were 7 guinea pigs getting one injection of tuberculin and seven injections of saline, 7 getting two injections of tuberculin and six of saline, and so on, the series ending with a set of 7 getting eight injections of tuberculin.

The responses to tuberculin on individual animals were averaged (when there was more than one), and the analysis of these data proceeded on the basis of a single response per guinea pig, using the procedure set out by Yates (8). This is an approximate procedure, justified by the relative magnitudes of the between- and within-guinea pig variances, whose ratio is about 5.5:1. The within-guinea pig component, which is inversely proportional to the number of tuberculin injections, thus only slightly affects the sum of the two components.

The sites of both the tuberculin and the saline injections showed varying degrees of erythema at twenty-four hours. At forty-eight hours, the erythema of the saline injections had virtually disappeared, leaving erythema only at the sites of tuberculin injection. In calculating the results, it has been assumed that the diameter of the reaction to tuberculin is not the sum of two responses, one to saline and one to tuberculin, but rather represents the effect of tuberculin independently of the effect of saline. In table 6, the mean results calculated from

TABLE 6

MEAN RESPONSE (DIAMETERS) TO i INJECTIONS OF TUBERCULIN

($i = 1, 2 \ldots 8$)

TIME READ AFTER INJECTION	NUMBER OF TUBERCULIN INJECTIONS							
	1	2	3	4	5	6	7	8
hours								
24	19.43	18.29	17.00	18.00	16.43	16.14	16.00	15.71
48	15.43	14.57	12.95	14.46	13.26	12.60	12.67	12.60

TABLE 7

MEAN RESPONSE (DIAMETERS) TO 2 γ OF PPD GIVEN IN 0.2 ML. OF NORMAL SALINE WHEN ASSOCIATED WITH DIFFERENT DOSES (IN 0.2 ML.) AT A SECOND SITE

ASSOCIATED DOSE OF PPD (γ)	2	4	6	8	10	12	14	16
Mean diameter of response (mm.)	16.6	18.0	14.4	15.8	13.8	7.6	10.4	12.2

the measurements at twenty-four hours after injection are shown, together with the forty-eight-hour measurement.

For purposes of comparison with the first experiment, the regression of area of response (diameter^2) on $\log^2 10$ (total dose) has been calculated and the resulting equation:

$$y^2 = 454 - 32 \log^2 10w$$

has a regression coefficient (-32 ± 1.33) which is remarkably close to the value of minus 26 found in the first experiment. This regression coefficient states the decrease in the area of response per unit increase in $\log^2 10w$. Clearly, when $\log^2 10w = \frac{454}{32} = 14.19$, the expected response to 2γ of PPD at another site on the same animal is zero. This gives $w = 590\gamma$.

It may be concluded that this experiment clearly demonstrates that increase in the number of identical injections on the guinea pig decreases the average response at all injection sites.

A further experiment was planned to determine whether the same depression effect was obtained when all the tuberculin was injected at a single site. In this experiment the argument tested was that the depression effect observed in previous experiments resulted essentially from the quantity of tuberculin injected and was not dependent on the number of reacting sites. In contrast to the previous experiment, therefore, the whole dose of tuberculin was divided between two sites, one of which had a constant dose (2γ of PPD in 0.2 ml. of normal saline) and the other i times this dose, where i could be any number between 1 and 8. Thus, in effect, this experiment tested the same quantities of tuberculin as the previous experiment.

A different randomization of the same balanced incomplete block design was used, in which a cage contained 4 guinea pigs, each getting one of the eight treatments. There were 7 animals in each treatment group, making 56 in all. The essential results are presented in table 7. It is clear that the depression effect, although present, is slight as compared with that recorded in the previous experiment, the regression obtained from equivalent calculations for two experiments being -32 ± 1.33 and -15 ± 4.88. There is thus a real difference between the degree of depression produced in the two experiments, and it seems reasonable to conclude that the depression in this third experiment has been mitigated because the variable dose, having been concentrated at a single site, causes much less erythema than when spread over a number of sites.

DISCUSSION

The results of assays conducted at four different levels of dosage indicate, by the significant differences between the PPD and OT dose-response curves, that qualitative differences exist between the two types of preparation. The differences are not large and cannot be accurately measured in small assays. This observation supports the inferences of other workers (1, 3) that at high concentrations the reaction to OT is exaggerated by substances other than the specific protein contained in the brew. These active materials may be glycerol, lipids, polysaccharides, evaporated broth, et cetera. Glover (9), for example, has found that heat-concentrated veal broth can by itself produce reaction simulating the tuberculin reaction. While such reactions may wholly or partly account for the difference in the dose-reponse curve of OT, the possibility remains that there may be qualitative differences in the specific proteins of OT and PPD; these differences would only be revealed in experiments with a PPD prepared from OT.

Since injections in the guinea pigs were randomized and the observer could work uninfluenced by the knowledge of which tuberculin had been injected at a particular site, an attempt was made to identify, by assessment of necrosis and intensity of erythema, which reactions were produced by OT and which by PPD. The tuberculins could not be differentiated on this basis in spite of the belief of the observer that reactions to OT tended to be more necrotic and severe than those to PPD. Great importance has been attached to qualitative appearance by several workers (10, 11), but such factors are difficult to evaluate numerically with any useful degree of precision.

The conclusion that the area of response rather than the diameter gives a straight line when plotted against log dose does not invalidate the results of previous workers (4*a*, 12, 13, 14) because a deviation from either relationship can be accurately measured only from a large-scale assay. The responses obtained by Chen and Sarber (20) had strong positive curvature, however, when area was plotted against log dose, and they found that this curvature was rectified when log area was plotted against log dose. Three points should be noted about that work: (*1*) the guinea pigs were sensitized with live organisms; (*2*) the responses obtained for equivalent doses were much less than those reported here; (*3*) doses were apparently not arranged in random order along the animal's back. Of these factors, the first and third are most likely to influence the shape of the dose-response curve.

Of the 8 smaller assays composing the large assay, only one (No. 4 of May 11, 1951) showed significant deviations of both slope and curvature (*see* table 3) although the combined effect, since the general tendency was in the same direction, was quite significant.

It can easily be shown (15, 16), by applying the principles of diffusion theory to agar plate assays of antimicrobials, that the square of the inhibition zone diameter should be proportional to the logarithm of the antimicrobial's concentration, and Humphrey and Lightbown (16) found that this relationship did, in fact, hold for a number of antimicrobials. Although a similar mathematical

relationship holds for intradermal tuberculin assays, one need not strictly infer that the diffusion constant is the factor regulating this dose-response curve. It does, however, seem worth while to make the inference and follow up the implications involved. One of the implications is that two tuberculoproteins which produce different dose-response curves should have different diffusion constants.

It has been stated that a skin reaction in allergic persons may accentuate neighboring reactions (17) or have no effect (18). It is difficult to design crucial experiments to determine if the depressing effect is a locally transmitted one or results from depression of the physiologic ability of the animal to respond. In the present study, it was not known whether the depression resulted from the large total quantity of tuberculin injected into the animal or whether it resulted from the large area of inflammation producing considerable stress and arising directly from the multiple injections. An injection of a constant amount of PPD was therefore associated with a second injection of increasing amounts of PPD in a replicated series of guinea pigs. Reactions associated with large dosage were only slightly smaller than those associated with small dosage, suggesting that dosage is not the main operative factor depressing response. In attempting to generalize, it is suggested that adjacent local reactions do not tend to influence one another without the intervention of a centrally stimulated mechanism. The present experiments were not designed to elucidate the physiology of the reaction depression, but it is conceivable that the phenomenon might be explained in terms of adrenal cortical stimulation. The final experiment is considered to be somewhat inconclusive, and more clear-cut results might have been obtained with wider spacing of the varying dose and a modified design. It would have been advisable to confirm the results of this experiment, but circumstances prevented the continuation of the work.

There appears to be no difference in assay results carried out after twenty-one days or sixty days in the same set of guinea pigs, and the slightly increased reactivity at the later test cannot certainly be attributed to either long duration of sensitization or to seasonal variation (19). It should be noted that one of the present writers (A.B.P.), using a different sample of OT, has had indications of an altered reactivity with time, using dead organisms in oil as the sensitizing agent.

SUMMARY

Differences in the dose-response curves of purified protein derivative and Old Tuberculin were demonstrated on guinea pigs sensitized with dead organisms in oil; such differences can be measured in only large-scale assays.

Similar results were obtained when the same experiment, using a fresh randomization scheme, was repeated after an interval of three months on the same set of sensitized guinea pigs.

Multiple tuberculin injections in the sensitized guinea pig depressed the response to the individual injection. The depression is probably related to the degree of systemic response and is not a local influence of one tuberculin reaction on an adjacent one.

SUMARIO

Factores que Afectan la Intradermorreacción a la Tuberculina en el Cobayo

En los cobayos sensibilizados con microbios muertos en aceite observáronse diferencias en las curvas de respuesta a la dosis de derivado proteínico purificado y de Tuberculina Antigua; esas diferencias no pueden medirse más que en valoraciones en gran escala.

Obtuviéronse resultados semejantes cuando se repitió el mismo experimento, usando un nuevo esquema de fortituidad, al cabo de un plazo de tres meses en la misma serie de cobayos sensibilizados.

Las inyecciones múltiples de tuberculina en los cobayos sensibilizados deprimieron la respuesta a la inyección dada. La depresión se relaciona probablemente con la intensidad de la respuesta orgánica general y no representa el influjo local de una reacción tuberculínica sobre otra adyacente.

RESUME

Facteurs affectant l'intradermo-réaction tuberculinique chez le cobaye

Les différences entre les courbes "dose-réaction" des tuberculines purifiées (PPD) et de la tuberculine de Koch (OT) ont été démontrées sur le cobaye sensibilisé avec des bacilles morts en solution huileuse; ces différences ne peuvent être mesurées que dans des déterminations effectuées sur une grande échelle.

Des résultats semblables ont été obtenus quand la même expérimentation a été répétée après un intervalle de trois mois sur la même série de cobayes sensibilisés, en utilisant un nouveau procédé de sélection au hasard.

Les injections multiples de tuberculine chez le cobaye sensibilisé provoquaient une réduction de la réaction à l'injection individuelle. Cette réduction est probablement en rapport avec le degré de la réaction générale organique; il ne s'agit pas de l'influence locale d'une réaction à la tuberculine sur une réaction contiguë.

Acknowledgment

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Case Reports

SELF-INOCULATION WITH M. TUBERCULOSIS AND PS. AERUGINOSA BY A DIABETIC WOMAN[1]

JAMES T. T. CHIEN AND MILTON L. WIGGINS

(Received for publication December 2, 1953)

Accidental cutaneous inoculation with tubercle bacilli in man is not rare, particularly among persons such as pathologists and meat handlers who frequently work with infected material. Deliberate self-inoculation with tubercle bacilli, however, either into the skin or intravenously by persons with suicidal intent, has rarely been reported. Only two instances of intravenous self-inoculation with tubercle bacilli could be found on examining the literature back to 1917. The most recent and best documented of these is the case reported by Jones and associates (1), in which a miliary pattern of dissemination in the lungs was demonstrated and in which the prompt use of antituberculous drugs resulted in apparent arrest of the disease.[2] The other case, reported by Lemierre and Ameuille (2), occurred prior to the advent of tuberculostatic drugs, and the patient died with widely disseminated tuberculosis within three months.

The present writers have not been able to find a reference to a previous instance in which intracutaneous or subcutaneous sites of self-inoculation were used. For this reason it seems worth while to report an unusual case of a diabetic and mentally disturbed young woman who administered suspensions of viable tubercle bacilli to herself via the skin of the left thigh, apparently with suicidal intent. An additional point of interest was the later inoculation, at an adjacent site, of material from a culture of *Ps. aeruginosa* (*B. pyocyaneus*) and the subsequent evolution of these two infections.

CASE REPORT

The patient, E. L., a 21-year-old single white female bacteriology technician, was admitted to the Psychiatric Division of Bellevue Hospital on February 4, 1952, complaining of a painful ulcer on the left thigh of one week's duration.

On January 5, 1952, the patient, apparently with suicidal intent, had injected 1 cc. of a suspension of viable tubercle bacilli subcutaneously into the proximal anterior aspect of her left thigh (figure 1, site *A*). This suspension, according to the patient, had been prepared by collecting the surface growth from a luxuriant four-week-old culture (Löwenstein medium) of *M. tuberculosis* with several swabs and soaking these swabs in a solution of crystalline insulin. The culture was derived from the sputum of a patient with active pulmonary tuberculosis. The patient of this report had repeated this procedure the following day, making the injection at a nearby site (figure 1, site *B*). A third injection, also into the left thigh (figure 1, site *C*), had been performed on January 12, 1952, this time with 1 cc. of a suspension prepared by washing trypticase soy broth over a second slant on which *M. tuberculosis* derived from the same patient mentioned above was growing.

On the day following each of these injections, an area of reddened, slightly painful swelling, 3 to 4 cm. in diameter, was noted by the patient at the injection site. These swellings

[1] From the Chest Service, First (Columbia University) Division of Bellevue Hospital, New York, New York.

[2] Patient known to be completely well at the present time; has been married and has a child (personal conversation between Dr. Jones and Editor).

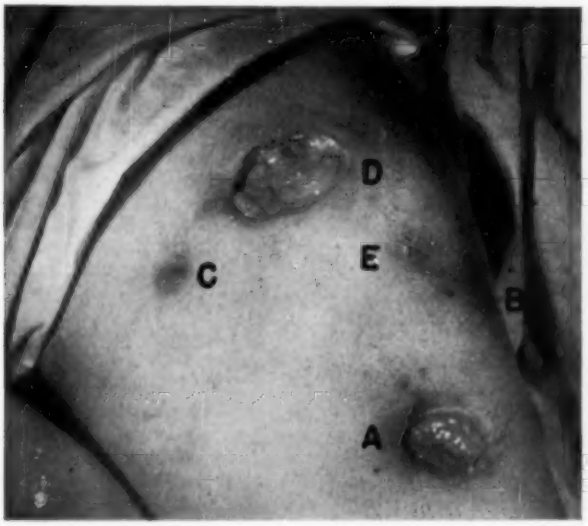

FIG. 1. The left thigh following self-inoculation with *M. tuberculosis* and *Ps. aeruginosa*. Sites *A*, *B*, *C*, and *E* are the result of the first, second, and third inoculations with *M. tuberculosis*. Site *D* is the result of inoculation with *Ps. aeruginosa*. The photograph was taken on the thirty-seventh hospital day (two weeks following spontaneous rupture of lesion *A* and incision of lesions *B* and *C*).

had gradually increased in size with some worsening of the pain; but no ulceration developed and there were no systemic manifestations.

Subsequently, on January 25, 1952, the patient had injected 1 cc. of a suspension of *Ps. aeruginosa* subcutaneously into an adjacent site on the left thigh (figure 1, site *D*). The day following this she developed headache and fever of 103° F. which persisted for two days. One week later the site of the *Ps. aeruginosa* inoculation began to ulcerate and, because of this, she was admitted to the hospital.

The patient had lived in New York City all of her life. A tuberculin skin test performed at the age of nine years had been negative, but this had not been repeated subsequently. Three years prior to admission the patient had been discovered to have diabetes mellitus. Her use of restricted diet and insulin had been sporadic and, because of this, her diabetes had never been under strict control for any period of time. On two previous occasions she had made half-hearted attempts at self-harm by using various biologic materials at her disposal, but these had been without deleterious effects. Because of these attempts she had been placed under private psychiatric care some months prior to the time of admission.

Physical examination on admission showed an obese white woman in no apparent distress. Her temperature was 100° F. (rectal), her pulse 90 per minute, and her respiration rate 20 per minute. The blood pressure was 122 systolic and 84 diastolic in mm. of mercury.

The skin generally was clear. However, there were five lesions over the proximal and anterior aspect of the left thigh. The first of these was a 5 cm. reddened, indurated area involving the skin and subcutaneous tissues, with a 4 cm. central ulceration which showed undermined margins and a grayish granular base. The other four lesions were roughly 2 to 3 cm. in diameter, red in color, firm and somewhat fluctuant, but with no ulceration. The sites of injection are illustrated in figure 1 which, however, shows them following spontaneous rupture of a tuberculous lesion (site *A*) and cross-incision of the other two tuber-

culous lesions (sites *B* and *C*). Lesion *E*, an area of induration which did not rupture spontaneously or require incision, was thought to be the result of subcutaneous inoculation at the time of injection at site *B*. In the left inguinal area there was a single, palpable, firm, slightly tender lymph node, 1 cm. in diameter. No nodes were palpable elsewhere. Examination of the head and neck was unremarkable. The lungs were clear on percussion and auscultation. The heart was normal in size with regular sinus rhythm, and no murmurs were heard. The abdomen was soft and obese, and there were no palpable organs or masses. Pelvic, rectal, and neurologic examinations did not reveal any abnormalities.

Laboratory examinations on admission revealed the hemoglobin to be 13.5 gm. per 100 cc. The erythrocyte count was 4.3 million per cu. mm.; and the leukocyte count, 15,000 with 72 per cent neutrophils, 27 per cent lymphocytes, and 1 per cent monocytes. The specific gravity of the urine was 1.022, and it contained a trace of albumin, a slight trace of a reducing substance, a trace of acetone, and 2 to 3 leukocytes per high-power field. A serologic test for syphilis (Wassermann) was negative. Determination of fasting blood sugar gave a value of 148 mg. per 100 cc. On March 6 the leukocyte count had fallen to 5,500, and another urinalysis on March 18 was normal. A culture of urinary sediment collected over twenty-four hours was negative for tubercle bacilli. A tuberculin patch test with activity equivalent to 0.1 mg. of Old Tuberculin was strongly positive. Three gastric washings were negative for tubercle bacilli on culture. A lumbar puncture revealed clear cerebrospinal fluid with only a few fresh erythrocyte cells. Analysis of this fluid revealed protein, 30 mg. per 100 cc.; glucose, 134 mg. per 100 cc.; and chlorides, 144 mEq. per liter. The cerebrospinal fluid culture was negative for tubercle bacilli. A routine roentgenogram of the chest on admission revealed no abnormalities of the pleura, lung, or mediastinum, and serial chest films taken subsequently have all been within normal limits.

Course in the hospital: Forty-eight hours after admission the patient was started on antituberculous therapy consisting of dihydrostreptomycin sulfate, 1 gm. intramuscularly daily, and sodium para-aminosalicylic acid (PAS), 12 gm. per day orally. Between March 20 and April 9, this medication was inadvertently discontinued but, on the latter date, therapy was reinstituted using dihydrostreptomycin sulfate, 1 gm. twice weekly, with daily PAS. This regimen was continued to September 8, 1952, when the patient was discharged.

At the time of admission, routine culture of the material from the ulcerated lesion of the the left thigh revealed *Ps. aeruginosa*. Antimicrobial treatment of the lesion was instituted, using 2 gm. of oxytetracycline (Terramycin) daily in divided doses for five days, followed by 2 gm. of chloramphenicol daily for a period of three weeks. During this therapy there was considerable healing of the large ulceration. On February 6, 1952, incision and drainage were performed on the two adjacent fluctuant areas. Following this procedure, drainage continued from the sites of incision. Cultures of the material obtained at time of incision were positive for *M. tuberculosis*. Biopsy of the lower medial ulcer was performed on February 15, 1952, and histologic examination of the specimen revealed multiple areas of tubercle formation (figure 2). These were both discrete and confluent with varying degrees of caseation necrosis. On March 12 an attempt was made to excise all the involved areas in two elliptical blocks with primary closure. The site of *Ps. aeruginosa* infection healed promptly by primary intention and has remained closed. However, the sites of excision of the tuberculous lesions reopened within four days, and again tubercle bacilli were evident in the discharged material. Subsequent local treatment consisted of enzymatic debridement with streptokinase and streptodornase. Following this the tuberculous wounds healed.

To summarize the treatment: On admission the patient was given 2 gm. of oxytetracycline daily for five days followed by 2 gm. daily of chloramphenicol for three weeks. In addition, on admission, daily doses of 1 gm. of dihydrostreptomycin sulfate and 12 gm. of PAS were initiated. This was continued for forty-three days, inadvertently discontinued, and then resumed on the sixty-third hospital day with the dose of dihydrostreptomycin sulfate reduced to 1 gm. twice weekly. Dihydrostreptomycin sulfate and PAS were then given for the remainder of her hospital stay until her discharge seven months after admis-

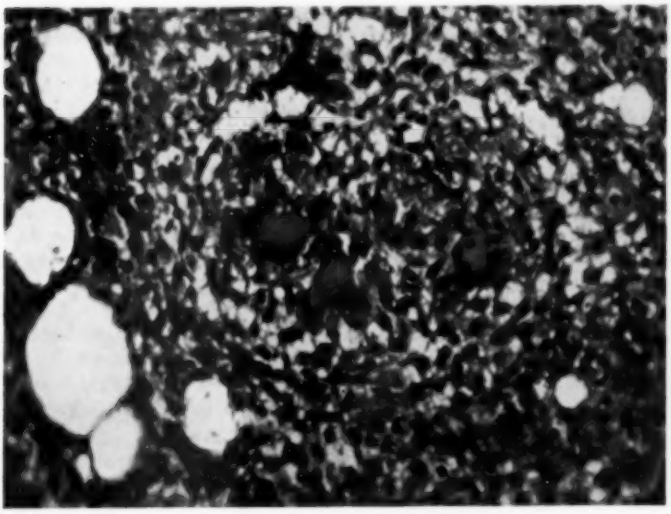

FIG. 2. An epithelioid and giant cell tubercle in the subcutaneous adipose tissue. Multiple discrete and confluent lesions of this type were present with necrosis in some. Tubercle bacilli were demonstrated in the tissue.

FIG. 3. Thigh wounds completely healed with residual scarring 16 months after excision.

sion. During this seven-month period of hospitalization, additional treatment in the form of surgical incision of the tuberculous lesions on the third hospital day, block excision of the tuberculous lesions on the thirty-seventh hospital day, and chemical debridement of postoperative draining sinuses during the fifteenth through the seventeenth hospital week were required. Complete closure was effected by the fifth month but, as noted above, antituberculous drug therapy was continued for another two months until discharge.

The patient's diabetes was easily brought under control with a dietary regimen and insulin. Throughout her hospital course there was no clinical, laboratory, or roentgenographic evidence of pulmonary or hematogenous tuberculosis. At the present time, one year after discharge from the hospital, the patient is entirely well and again employed as a laboratory technician.

COMMENT

Although it cannot be established with certainty that this patient had had a sensitizing infection with *M. tuberculosis* prior to the present episode, the behavior of the sites of inoculation and the absence of active tuberculosis developing elsewhere in the body is suggestive that she was indeed a tuberculin reactor prior to the bacillary inoculation. The local accelerated and exaggerated reaction and the absence of definite regional node involvement is reminiscent of Koch's description of the so-called "Koch phenomenon" elicited by cutaneous reinfection of tuberculous guinea pigs (3). The inhibition of hematogenous dissemination in these reinfected animals was then demonstrated by Römer (4) and Hamburger (5). Mishulow and Park (6), Krause (7), Willis (8, 9), Lurie (10), and others have similarly shown that, in an animal which has not previously been sensitized by exposure to tubercle bacilli, there will be rapid lymphohematogenous dissemination from a site of inoculation with these organisms. On the other hand, in previously sensitized animals, a subsequent injection of virulent organisms will result in a severe local reaction with sloughing but with little immediate evidence of spread to the regional lymph nodes or to other organs. If, under these conditions, evidence of lymphohematogenous dissemination becomes apparent at a later time, the extent of the regional lymphatic and disseminated disease is rarely so great as that seen in the nonsensitized animal.

If an analogy can be drawn between these observations and human tuberculosis, the fact that the patient reported here developed a frankly necrotic lesion at the site of *M. tuberculosis* inoculation with only equivocal regional adenopathy and no evidence of hematogenous dissemination would suggest that she had previously coped successfully with a natural tuberculous infection.

It is of interest that it was not until the patient had infected herself with *Ps. aeruginosa* that her local and systemic reactions became sufficiently severe to necessitate hospitalization. In contrast, however, the *Pseudomonas* infection was readily brought under control with antimicrobials and simple excision, whereas the local tuberculous lesions showed evidence of much longer activity despite streptomycin and PAS therapy, excisional procedure, and the use of fibrinolytic enzymes.

SUMMARY

A case of self-induced tuberculous and *Pseudomonas* (*B. Pyocyaneus*) subcutaneous abscesses in a young female diabetic patient is presented. The differences in the symptoms, the local developments, and the response to treatment are described. The relationship of the local and systemic manifestations to possible prior natural tuberculous infection is discussed, and the methods of treatment employed in this case are described.

SUMARIO

Autoinoculación de Bacilos Tuberculosos y Ps. aeruginosa (B. Pyocyaneus) por una Joven Diabética

Preséntase un caso de abscesos subcutáneos tuberculosos y piociánicos autoprovocados en una joven diabética. Descríbense las diferencias en los síntomas, los fenómenos locales y la respuesta al tratamiento. Discútese la relación de las manifestaciones locales y consti-

tucionales con una posible infección tuberculosa natural anterior y se describen los métodos de tratamiento empleados en este caso.

RESUME

Auto-inoculation avec des bacilles tuberculeux et des Ps. Aeruginosa (B. Pyocyaneus) par une jeune femme diabétique

Présentation d'un cas d'abcès sous-cutanés tuberculeux et pyocyaniques par auto-inoculation chez une jeune femme diabétique. Les différences dans les symptômes, l'évolution locale et la réponse au traitement sont décrites. La relation entre les manifestations locales et générales et une affection tuberculeuse antérieure possible est commentée. Description des traitements employés dans ce cas.

Acknowledgment

The authors wish to express their gratitude to Dr. J. Burns Amberson for his review of the material and his invaluable advice.

The authors are also indebted to Dr. Marvin Kuschner, assistant pathologist, Bellevue Hospital, and assistant professor of pathology, College of Physicians & Surgeons, Columbia University, New York, New York, for his very kind assistance.

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TOXIC REACTION TO PARA-AMINOSALICYLIC ACID AND ISONIAZID ACCOMPANIED BY LEUKOPENIA AND ATYPICAL LYMPHOCYTOSIS[1]

SUSAN J. MELLETTE AND HARRY AGRESS

(Received for publication October 5, 1953)

Serious toxic reactions to para-aminosalicylic acid (PAS) or to isoniazid as used in the treatment of tuberculosis have been relatively uncommon. Allergic or hypersensitivity reactions occasionally occur and include fever, chills, various skin rashes, and, more rarely, hematologic manifestations (1, 2). The development of a syndrome closely resembling the clinical picture of acute infectious mononucleosis has been reported following the administration of PAS (3). This syndrome occurred in the patient to be described below while she was receiving both PAS and isoniazid and was reproduced by test doses of each of these drugs given separately. So far as is known, the development of this condition as a complication of isoniazid therapy has not previously been reported.

CASE REPORT

M. J., a 28-year-old white married female, was admitted to Robert Koch Hospital on March 10, 1953. She gave a history of cough, intermittent fever, and progressive weight loss, beginning approximately four months prior to admission. She had received penicillin and sulfonamide therapy without improvement.

Except for attacks of "pleurisy," lasting approximately two weeks, at ages sixteen and nineteen and an appendectomy at nineteen, the patient had not been ill previously. The past history was otherwise noncontributory. Neither the patient nor members of her family had suffered from urticaria, hay fever, asthma, or other allergic manifestations.

Physical examination revealed a well-developed and well-nourished young woman who was apprehensive but did not appear ill. A few firm pea-sized lymph nodes were present in the left cervical and axillary areas. Post-tussic rales, bronchovesicular breath sounds, and some decreased resonance to percussion over the left upper and mid chest were noted. There were no other abnormal findings. The liver and spleen were not palpable.

Roentgenograms of the chest revealed a moderately dense scattered infiltration involving the entire left lung field with the exception of the base. Sputum was positive for acid-fast bacilli.

The leukocyte count on admission was 12,650 per cu. mm. with a Schilling differential as follows: basophils, 0; eosinophiles, 3; myelocytes, 0; juveniles, 0; stab cells, 12; segmented neutrophiles, 70; lymphocytes, 8; and monocytes, 7. The erythrocyte count was 4,200,000 per cu. mm. and hemoglobin 14.3 gm. per 100 cc. Urinalysis was essentially normal.

The diagnosis was pulmonary tuberculosis, far advanced and active.

Course in hospital: The patient continued to have intermittent fever with peaks of about 100° F. for the first week after admission. This subsided gradually to normal levels. On March 23, treatment with sodium para-aminosalicylate (PAS), 12 gm. daily in four divided doses, and isoniazid, 550 mg. (10 mg. per kg. of body weight) daily in two divided doses, was begun. This regimen had been outlined by the Public Health Service for investigative purposes.

During the afternoon of the first day of treatment, the patient complained of flushing of the face and chilliness. These symptoms subsided spontaneously, and treatment was continued without incident until April 7, sixteen days after the drugs had been begun. At this time, the patient again complained of feeling "flushed." No fever was present. A few doses of antihistaminics were given during the next three days for persistent similar symp-

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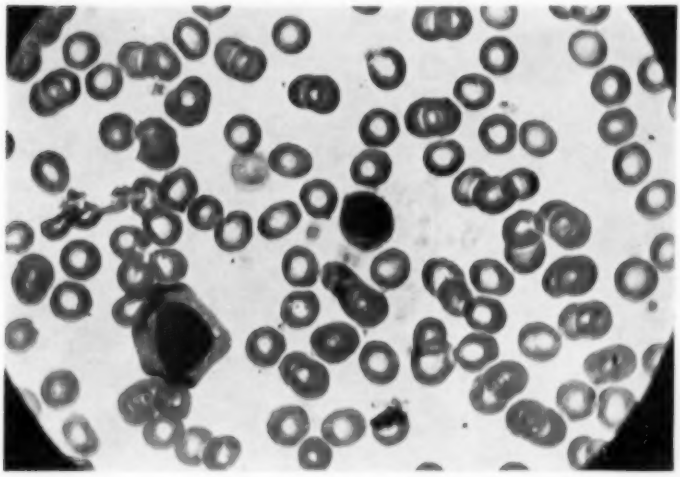

FIG. 1. Photomicrograph (×2,000) of peripheral blood spread of patient M. J. The large lymphocyte in the right upper portion of the field shows marked vacuolization of the cytoplasm. Note the dark concentration of staining about the periphery of this cell (separation of spongioplasm and hyaloplasm). The smaller lymphocyte in the center of the field is relatively normal except for a few vacuoles in the cytoplasm.

toms; but fever began to rise, reaching a peak of 103° F. with chill on April 12. PAS was discontinued.

The following day, the patient's temperature remained elevated and continued to climb to a level of 103.6° F. Blood counts revealed a leukopenia of 3,900 leukocytes per cu. mm. Isoniazid was also discontinued. The patient's temperature promptly returned to normal overnight and remained normal. About two days later, a maculopapular, mildly pruritic rash appeared over the face, neck, and shoulders. The rash persisted for approximately three days, leaving petechiae and purpuric blotches as it faded. Moderate, slightly tender, cervical and axillary adenopathy also developed concurrently, but neither the liver nor the spleen could be palpated.

The leukocyte count, meanwhile, fell to 3,500 per cu. mm. on the day after the discontinuation of isoniazid; then climbed within four days to 10,000, with the development of a lymphocytosis reaching 64 per cent. Nearly all of the lymphocytes at this stage were large atypical cells with irregularly staining, vacuolated cytoplasm of the type commonly associated with acute infectious mononucleosis (figure 1). Heterophile agglutinations performed on three separate occasions over a period of three weeks were negative.

Erythrocyte counts and hemoglobin determinations remained essentially normal. An adequate number of platelets was consistently present on spreads of the peripheral blood. A bone marrow aspiration performed on April 14, at the height of the leukopenia, revealed a slight shift to the left of the myeloid elements and a slight increase in plasma cells, but was otherwise not remarkable.

On April 29, after the blood counts had returned to normal and abnormal cells had virtually disappeared from the spreads, a test dose of 250 mg. of isoniazid was given. The patient's temperature rose to 102.6° F. within six hours. The following day, the leukocyte count had fallen from its previous level of around 7,000 to 5,200 per cu. mm. Again, the count rapidly increased, with lymphocytosis and a high percentage of atypical lymphocytes appearing on the fourth day.

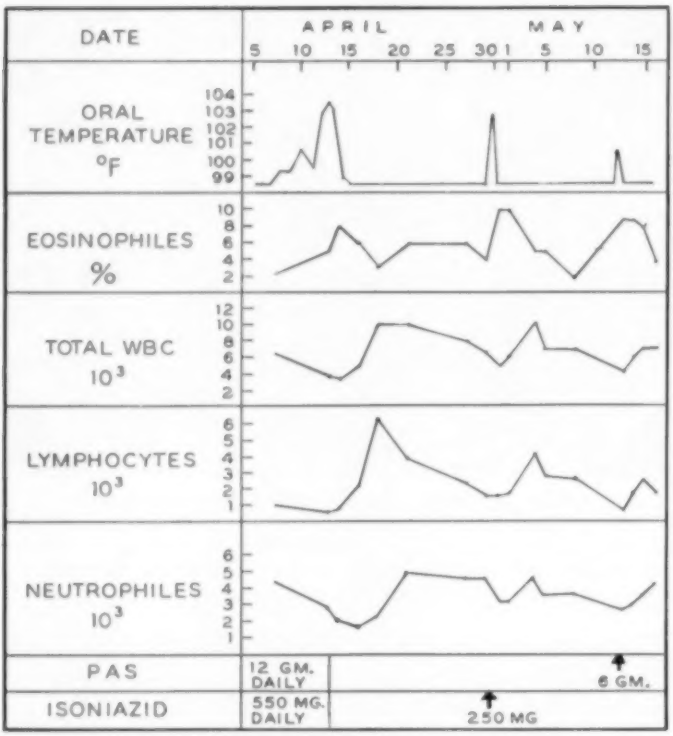

FIG. 2. Chart showing blood counts and temperature curve during initial episode and following test doses of para-aminosalicylic acid and isoniazid.

On May 12, after the blood studies were again normal, and after a 1 gm. dose of PAS had been given on May 5 without reaction, PAS was again begun. After two doses (3 gm. each) had been given, fever again occurred. Again, slight leukopenia (4,600 per cu. mm.) developed, followed by a rising count with an increasing percentage of lymphocytes and the reappearance of distinctly atypical lymphocytes. Blood counts and temperature curve during the initial episode and following the test doses of PAS and isoniazid are demonstrated in figure 2.

A first attempt at "desensitization" with PAS was carried out, beginning May 21; but the drug was again discontinued ten days later because of the reappearance of pruritic skin rash. A dosage level of 8 gm. of PAS daily had been reached.

Ten days later isoniazid was resumed, beginning with a 25 mg. dose daily and increasing gradually over a period of several weeks until the full dosage of 550 mg. daily was reached. PAS was then added, beginning with 250 mg. and also slowly increasing until 12 gm. daily was well tolerated by the patient.

Increases in dosage were determined by following the leukocyte counts and differential spreads, as well as the patient's clinical condition. If the patient complained of afternoon flushing, if more than moderate eosinophilia occurred, or if a significant number of vacuolated or irregularly staining lymphocytes appeared, the dosage was retained or a dose or two omitted. No significant trends in total or differential counts occurred under these condi-

tions; and, although the lymphocytes sometimes showed slight "toxic changes," there was no reappearance of a significant number of the large atypical cells. The patient's temperature remained essentially normal during this period.

Test doses of sulfadiazine (1.0 gm.) and acetylsalicylic acid (0.6 gm.) were given without reaction. Skin tests to 3 per cent of PAS and to 3 per cent of isoniazid in petrolatum yielded negative results.

COMMENT

It is believed that leukopenia, often followed by lymphocytosis, as a reaction to either PAS or isoniazid is probably a more common phenomenon than is usually recognized.

In the patient described, and in several other patients exhibiting febrile reactions to either PAS or isoniazid given separately, the sequence of hematologic changes is as follows:

1. An inconstant and transitory leukocytosis with a shift to the left of the neutrophiles associated with the febrile reaction itself
2. Leukopenia, with an essentially normal differential count or slight neutropenia
3. A gradually increasing total leukocyte count accompanied by relative and absolute increase in lymphocytes with the appearance of atypical forms

If blood counts are not done almost daily, phases of this pattern will obviously be overlooked, and the results of isolated counts may be misinterpreted. It is recommended, therefore, that, during any attempt to reinstitute therapy after a severe allergic drug reaction, careful watch be kept on the hematologic picture.

The abnormal lymphocytes described have been reported in various other conditions, such as infectious mononucleosis, infectious hepatitis, and other viral diseases (4). Similar cells have also been reported in allergic conditions (5), but in none of the cases in this report did the number of lymphocytes exceed that of the neutrophiles.

It is of interest that the present writers have seen a few abnormal lymphocytes similar to those described occasionally in routine spreads of the blood of patients who received either PAS or isoniazid or both drugs, even in the absence of fever or other clinical signs of drug sensitivity. The difference is one of degree, both in the number of such cells present and in the amount of vacuolization or abnormal staining of the nucleus and cytoplasm. Some of these patients also demonstrate a higher than anticipated percentage of lymphocytes in peripheral blood spreads.

SUMMARY

A patient exhibiting toxic reactions to both PAS and isoniazid has been described. In addition to the clinical manifestations, hematologic changes occurred. There was the development of moderate leukopenia followed by lymphocytosis with the appearance of abnormal lymphocytes of the type usually associated with acute infectious mononucleosis. These changes which occurred during therapy were reproduced by test doses of PAS and isoniazid administered separately.

SUMARIO

Reacción Tóxica Tanto al Ácido Para-Aminosalicílico como a la Isoniacida, Acompañada de Leucopenia y de Linfocitosis Atípica

Descríbese un caso que manifestó reacciones tóxicas al ácido para-aminosalicílico (PAS) a la vez que a la isoniacida. Además de las manifestaciones clínicas, hubo alteraciones hematológicas. Se presentó leucopenia moderada seguida de linfocitosis con la aparición de linfocitos anormales de la forma habitualmente asociada con mononucleosis infecciosa

aguda. Esas alteraciones que tuvieron lugar durante la terapéutica fueron reproducidas con dosis experimentales de PAS y de isoniacida, administrados por separado.

RESUME

Réaction d'intolérance à la fois pour l'acide para-amino-salicylique et pour l'isoniazide accompagnée de leucopénie et de lymphocytose atypique

Un cas présentant des réactions toxiques à la fois à l'acide para-amino-salicylique (PAS) et à l'isoniazide a été décrit; outre les manifestations cliniques il s'est produit des modifications hématologiques. Une leucopénie modérée suivie de lymphocytose s'est manifestée avec apparition de lymphocytes anormaux, du type habituellement associé à la mononucléose infectieuse aiguë. Ces altérations, qui se sont produites au cours du traitement, ont pu être reproduites par des doses d'épreuve de PAS et d'isoniazide administrées séparément.

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THE SURGICAL SIGNIFICANCE OF SOLITARY CIRCUMSCRIBED LUNG NODULES IN HISTOPLASMOSIS[1]

Report of Three Cases

DON E. MATTHIESEN[2]

(Received for publication October 5, 1953)

The attention given to so-called pulmonary coin lesions in recent years has yielded dividends considerably beyond early expectations. The number of patients with primary lung malignancies whose lives have been saved by prompt surgical intervention following discovery of such lesions has been significant. A not inconsiderable group of solitary metastases have been similarly removed with great benefit, and in some cases with apparent cure. In addition, the surgical resection of solitary benign pulmonary lesions of various types has proved of value. In general, the medical profession has widely accepted and approved the philosophy of early removal of solitary, circumscribed pulmonary lesions. Nevertheless, the problems attending preoperative diagnosis of such lesions have not been worked out as satisfactorily. Postoperative histologic examination has come to be almost the sole acceptable method of appraisal. The few meager diagnostic clues which have been commonly used are in need of revision; and even pathologic study may at times be misleading.

Solitary pulmonary nodules probably occur more frequently in histoplasmosis infections than has heretofore been recognized, as the following abstracted case reports suggest.

These three conspicuously similar tumors recently, within a period of a few days, have come to the attention of the writer; and their features are such that it is believed that they are worthy of report.

CASE REPORTS

The first case was that of a 38-year-old housewife who had, during the past several years, lived in Florida, Washington, Tennessee, and Alaska. She was admitted without symptoms for repeat roentgenographic examination of a solitary circumscribed nodule in the base of the left lung. This was known to have been present for two or three years, and had been followed with serial roentgenograms for nine months with no evidence of change.

The general physical examination and routine laboratory findings on the patient were not remarkably abnormal at the time of admission. Examinations of other systems were negative. A histoplasmin skin test was strongly positive. Tuberculin skin test was also positive. On chest roentgenograms, a sharply circumscribed nodule, 1.5 cm. in diameter, was seen in the peripheral portion of the left lower lung field. Definite visualization of calcium within the lesion was not possible. Surgical resection of the lesion was performed, and frozen-section examination revealed the lesion to be a granuloma caused by *Histoplasma capsulatum*. A swab obtained from the lesion showed the organisms on microscopic examination, and later cultures verified the presence of the organisms.

Grossly, the lesion was firm and discrete, but not encapsulated. Cut section showed mottled, partly laminated, whitish-tan to dark blue caseous material with no evidence of necrosis. Microscopic study showed a thin layer of lung which showed some inflammatory infiltration. There was a thin layer of granulation tissue, and the remainder and major portion consisted of homogeneous caseous material. The typical fungi characteristic of *Histoplasma capsulatum*, some showing endospores with surrounding torulation, were seen.

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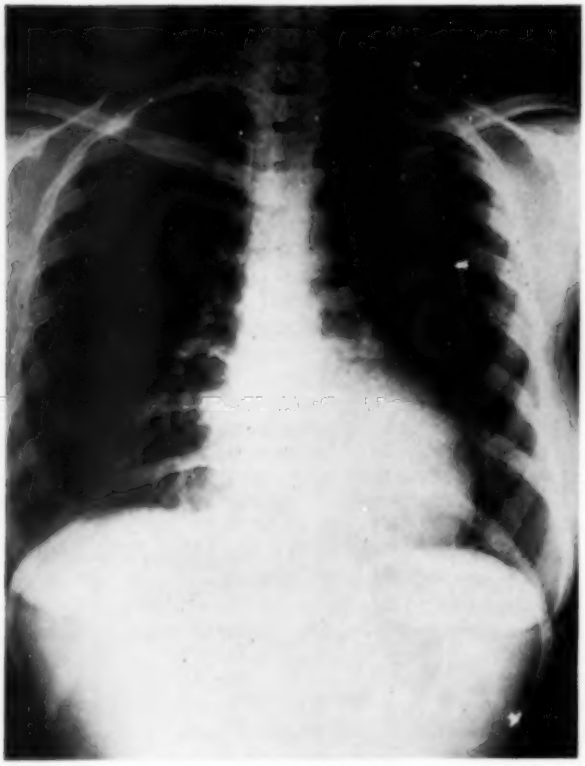

FIG. 1. Case 1. Roentgenogram showing rounded nodule in left sixth anterior interspace.

From the appearance of the wet preparation of the swab, this would seem to have been a fairly active lesion.

The second case was that of a 24-year-old male who had lived on the Atlantic seaboard throughout his lifetime. He was admitted without symptoms for investigation of a partially calcified nodular lesion found in his left lung on a routine roentgenographic examination of the chest. Previous chest films had all been reported normal, but none could be obtained for comparative study. The patient's history, general physical examination, and admission laboratory work were completely normal. A skin test with first-strength purified protein derivative (PPD) was negative, but the reaction to second-strength PPD was strongly positive. A coccidioidin skin test was negative. A histoplasmin skin test was strongly positive. In addition, sputum studies on this patient revealed *Histoplasma capsulatum*. Roentgenographic examination revealed a round, sharply circumscribed lesion, 2 cm. in diameter, in the left lower mid-lung field. Its outer border had a faintly laminated appearance, its central zone was irregularly calcified, and a zone of relative radiolucency lay between. The lung lesion was removed surgically, and the gross features of the lesion were strikingly similar to those in the first case reported above. Microscopically, the appearance of lami-

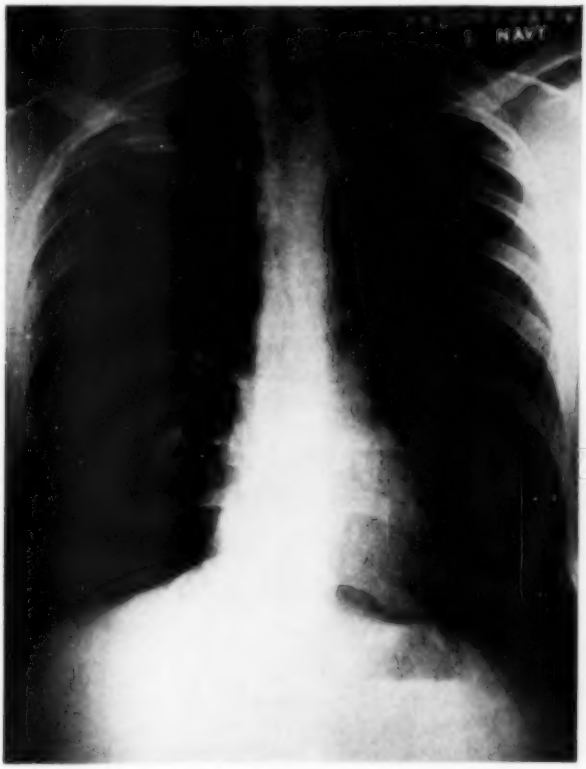

FIG. 2. Case 2. Roentgenogram showing lesion with laminated calcifications beneath anterior end of left sixth rib.

nated calcification was verified. This lesion also showed the organisms of histoplasmosis on wet smear, permanent preparations, and on cultures.

The third case was that of a 32-year-old male who was told four months prior to admission that there was a "coin" lesion in his left lung. Previous roentgenograms were said to have been normal but could not be obtained for comparative study. Two months before the "coin" lesion was first reported, the patient had had "flu" and a cough for a few days, after which time he had been completely well and free from symptoms. His history included residence in several foreign countries and in numerous places in the United States. Physical examination and routine admission laboratory work were negative. First- and second-strength PPD skin tests were negative. Coccidioidin skin test was negative. Histoplasmin skin test was strongly positive. Sputum studies were negative for fungi or acid-fast bacilli. Roentgenographic studies of his lungs showed an oval opacity of rather sharp outline which measured 1.5 by 1 cm. and was situated in the region of the lateral basal segment of the left lower lobe. Bucky films suggested the presence of finely mottled calcifications in the lesion. The lesion was removed at surgery without incident, and grossly was described as a dis-

FIG. 3. Case 3. Roentgenogram showing "coin" lesion beneath left fifth rib anteriorly.

crete but apparently not encapsulated lesion 1.3 cm. in diameter. On cut section there was a feeling of calcium salts. The resemblance of this lesion to that of *Case 1* above was remarkable. On microscopic study the presence of *Histoplasma capsulatum* was established both on frozen section and smear from the lesion and on permanent slides.

COMMENT

Many excellent reviews are to be found in recent medical literature which take up fully the various aspects of histoplasmosis. It is believed, however, that the implications of the findings in the cases above described are significant and that a brief discussion of them is not out of order.

First, three cases in a row on one service would seem to indicate that solitary lung nodules in histoplasmosis infections are not rare, despite suggestions to the contrary in current medical literature. Second, the diagnostic features upon which principal reliance has been placed in appraising such lesions seem to be undependable; and it is not unlikely that the granulomata of histoplasmosis have been misdiagnosed many times in the past. Histoplasmosis should be added to the list of diagnostic possibilities in the consideration of solitary pulmonary coin lesions. Third, the present experience confirms that of others, that surgical resection of pulmonary lesions is evidently good treatment in the management of such localized histoplasmosis infections.

Evidence has accumulated in recent years to support the concept of a benign form of histoplasmosis as a clinical entity. There has been little information, however, to suggest that this form of the disease produces solitary pulmonary nodules of the type observed in the cases here reported. Numerous series have indicated that multiple nodules are common in this disease, and that they are very often associated with hilar adenopathy and not infrequently with lymphoblastoma. Large series of pulmonary "coin" lesions surgically removed have been reported in the past five years; and it is interesting to note that, although the incidence of granulomata has been reported as high as 70 per cent, the etiology has rarely been suspected or proved to be histoplasmosis.

In many instances the finding of a grossly fibrocaseous lesion at surgery has been the only basis upon which the diagnosis of tuberculoma has been made. Since most such lesions have been anatomically situated away from the lung apices, it has been suggested that, if a tuberculous focus is located in an atypical site, it may be more likely to assume the form of a tuberculoma. This notion is probably erroneous, and it is more likely that such granulomas are very often not tuberculous. The various investigators who have written on the subject of pulmonary granulomas have nearly all reported that only exceptionally has it been possible to establish tuberculosis unequivocally. As a matter of fact, only 3 of 65 lung granulomas in one series had even a dubious history of tuberculosis. One writer has stated that 25 per cent of patients with tuberculoma are negative cutaneous reactors to tuberculin, which seems to support the probability of frequent nontuberculous etiology in pulmonary granulomata.

Diagnostically, the presence of calcium on roentgenographic examination does not warrant unlimited assurance, although it is thought to suggest a benign process in most cases. Some lesions with relatively high calcium concentrations may be soft and caseous and, therefore, capable of unfavorable development. Calcium in a lung nodule has been described in a metastasis from osteogenic sarcoma. The principle that calcification is more likely to signify the presence of tuberculoma is open to question; and more studies to rule out etiologic agents other than tubercle bacilli are indicated.

On the other hand, the lack of calcium in a pulmonary nodule does not necessarily indicate a malignant process. Less than half of the reported lesions of this type have proved malignant. There is a limit to the amount of calcium which can be demonstrated roentgenographically through the tissue of the chest wall; and many lesions, apparently without calcium on roentgenographic examination, contain large amounts histologically.

Multiple areas of diminished density in a calcified lesion are thought to represent areas of caseation and fibrosis, and may suggest not only tuberculoma but other types of granulomata as well. These rarefied areas may act as foci of a spreading disease process and thus may contraindicate a policy of delay. Calcium, if not laid down in a solid, uniform manner, probably should be considered an indication for prompt surgery.

Solitary pulmonary nodules apparently cannot be differentiated on a basis of size, shape, situation, or growth characteristics with the present state of knowledge. The almost identical location anatomically of the lesions in the cases reported above may prove later to have some significance.

Pathologically, the occasional close resemblance of granulomata on microscopic examination to anaplastic malignant neoplasm has been observed and, in the absence of positive identification of causal organisms, it may be very difficult to distinguish between the two.

Some investigators believe that an infectious granuloma with neoplastic tendencies may develop secondary to chronic hyperplastic inflammation. Because of the varied histologic pictures which are seen, the cell of origin is the subject of rather wide differences of opinion.

Evidently the process tends initially to be principally inflammatory, with dilatation of lymphatics and blood vessels. This is accompanied by perivascular edema, hemorrhage, and infiltration of plasma cells, round cells, and connective tissue cells. Later the proliferation of connective tissue and small vessels is added so that the granulomatous features seem to predominate. Still later, the neoplastic features are noted, the picture depending upon whether the vascular or connective tissue elements are most marked.

The suggestion then seems to follow logically that perhaps all "coin" lesions diagnosed histologically as malignant tumor deserve intensive study for *Histoplasma capsulatum*. It is not unlikely that in occasional instances a resected lung lesion will prove more benign than initial microscopic examination might suggest. In addition, difficulties in differentiation are to be anticipated at times in separating these granulomas from tuberculosis and syphilis.

Histoplasmosis has been thought to have a tendency to disseminate so that it is not often sufficiently localized to permit surgical resection. The experience in these three cases, however, would seem to imply that it may tend otherwise. As pointed out by Hodgson, Weed, and Clagett (11), whenever feasible, excision of the disease is probably good treatment. It is true that in some cases the disease is so protracted that it is difficult to know whether the patient is cured or not, but apparent continued clinical well-being is probably sufficiently good evidence to permit the assumption that the disease has been inactivated.

The weak spot in the treatment of solitary pulmonary nodules is the period of time which elapses between its discovery and the application of appropriate treatment. It also appears that unfounded assumptions may be made at times concerning the nature and etiology of such nodules. Without complete investigation, it should not be the practice simply to label all lesions containing calcium as tuberculomas or all relatively undifferentiated granulomatous tissue as a malignant process.

As the present state of knowledge will not permit reliance upon roentgenographic findings for diagnosis in these cases, it becomes the prime duty of the physician to advise the patient of the presence of a roentgenographic shadow, the exact nature of which cannot be established without operation. He should then strongly urge the surgical removal of the lesion because of the possibility of malignancy. If this type of management can be carried through to its logical conclusion and then careful histologic search with appropriate techniques be made of tissue sections, it is almost certain that many errors will be avoided in future.

SUMMARY

Solitary, circumscribed pulmonary lesions occurring in histoplasmosis infections are reported in 3 cases. It is suggested that *Histoplasma capsulatum* has probably been responsible in many cases for granulomata reported in the past as due to tubercle bacilli. Histoplasmosis should be added to the list of considerations in differential diagnosis of solitary pulmonary lesions. The diagnostic implications of the presence of calcium in pulmonary "coin" lesions are reviewed. It is pointed out that at times the presence of calcification is actually an indication for surgical resection. The microscopic resemblance of the granulomata of *Histoplasma capsulatum* to tuberculosis, syphilis, and anaplastic lung cancer is noted.

The therapeutic value of surgical resection of such disease is being observed more frequently than heretofore. It is to be expected that, as histoplasmosis is suspected and searched for more in the future, it will be found that the number of cases suitable for surgery is higher than was previously believed.

SUMARIO

La Importancia Quirúrgica de Nódulos Pulmonares Circunscritos Solitarios en la Histoplasmosis. Presentación de Tres Casos

Descríbense 3 casos de lesiones pulmonares circunscritas solitarias que fueron observadas en la histoplasmosis. Sugiérese que el *Histoplasma capsulatum* ha sido probablemente la causa de muchos casos de granuloma atribuídos en el pasado al bacilo tuberculoso. Hay que agregar la histoplasmosis a la lista de los factores por considerar en el diagnóstico diferencial. Repásanse las inferencias diagnósticas a que conduce la presencia de calcio en lesiones pulmonares "en moneda." Señálase que a veces la calcificación indica en realidad la resección quirúrgica. Nótase la semejanza microscópica de los granulomas del *Histoplasma capsulatum* con la tuberculosis, la sífilis y el cáncer pulmonar y anaplásico.

Con más frecuencia que jamás antes obsérvase el valor terapéutico de la resección quirúrgica en esa enfermedad. Es de esperar que, a medida que se sospecha y busca más la histoplasmosis en el futuro, se descubrirá que el número de casos apropiados para la cirugía es más alto que lo que se creía antes.

RESUME

Signification chirurgicale des nodules solitaires circonscrits du poumon dans l'histoplasmose: Rapport sur trois cas

Trois observations de lésions pulmonaires solitaires et circonscrites qui se sont produite dans l'histoplasmose sont présentées. L'auteur suppose que, dans de nombreux cas, l'*Histoplasma capsulatum* a été probablement responsable des granulomes attribués jadis au bacille tuberculeux. L'histoplasmose devrait être ajoutée à la liste des affections à considérer dans le diagnostic différentiel. Les implications diagnostiques de la présence de calcium dans les lésions arrondies du poumon sont passées en revue. La présence d'une calcification est signalée comme étant parfois une indication de résection chirurgicale. La ressemblance microscopique du granulome de l'*Histoplasma capsulatum* avec la tuberculose, la syphilis et les tumeurs malignes anaplastiques du poumon est mentionnée.

La valeur thérapeutique de la résection chirurgicale dans cette affection est observée plus fréquemment qu'autrefois. On peut prévoir que, dans l'avenir, et dans la mesure où le diagnostic d'histoplasmose sera considéré et recherché, on découvrira que le nombre de cas justiciables de la chirurgie est plus élevé qu'on ne le pensait.

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Notes

RESULTS OF MASS ROENTGENOGRAPHY AMONG IMMIGRANTS INTO ISRAEL

After the inauguration of the state of Israel in 1948, a mass immigration of Jews from Europe, Asia, and North Africa started and, until the end of 1951, approximately 700,000 immigrants were registered. Nearly 300,000 persons were examined by mass roentgenography, and a relatively high percentage of active tuberculosis was detected. The morbidity rate varied remarkably in the course of time: 1948–1949, 0.8 per cent; 1950, 0.54 per cent; 1951, 0.3 per cent.

In search for an explanation of these differences, an analysis of the material was made with regard to sex, age, and the countries of origin of the immigrants. It was found that the different epidemiologic conditions in the countries of origin accounted for the different tuberculosis morbidity rates.

It was observed that in each of the four years considerable numbers migrated from certain countries. In 1949, and in 1948 as well, the flow of immigrants brought the remaining Jews from the concentration camps in Europe. The tuberculosis morbidity among them was very high. The year 1949 was also marked by immigration from Yemen and Turkey. The immigrants from Turkey, although representing only 10 per cent of the total number of immigrants, account for 30 per cent of the cases of active tuberculosis in 1949 (figure 1).

In figure 1, only the European countries with especially great migration to Israel are listed, such as Poland, Roumania, and Czechoslovakia. In addition, Turkey is listed, where the extreme disproportion between the immigration rate and the morbidity rate is clearly demonstrated.

The high percentage of active tuberculosis (0.8 per cent) in 1949 is understandable because of the unfortunate condition of the European Jews and the exceptionally high morbidity among the immigrants from Turkey.

In 1950, the immigration from Arabic countries and Persia became noteworthy. The tuberculosis morbidity in the Arabic countries is notoriously low, and this is reflected in the epidemiologic condition among the Jews who came from there. Therefore, notwithstanding the high percentage of active tuberculosis among those who came from Europe, the average morbidity rate in 1950 was lower because of the lesser incidence of tuberculosis among the Oriental Jews.

The immigrants from Poland and Roumania had a relatively greater share in the tuberculosis morbidity than would correspond to their part in the total immigration, as may be seen in figure 2. An inverse proportion may be noted with regard to the Oriental Jews. On the other hand, among the European Jews, less cases of primary infection are registered than among the Jews from Iraq, Persia, and North Africa.

The migration from Turkey in 1950 was not noteworthy in quantity, but the morbidity rate was, as it was in 1949, rather high.

In 1951, there were 174,706 newcomers to Israel. One hundred and sixteen thousand of them immigrated from Arabic countries. It is evident that, because of the low morbidity rate among such a large proportion, the average percentage of active tuberculosis became still lower than in the previous year, namely 0.3 per cent.

The relation between immigration rate and morbidity rate with regard to the different countries of origin in 1951 is illustrated in figure 3. As in 1950 (figure 2), there was a high

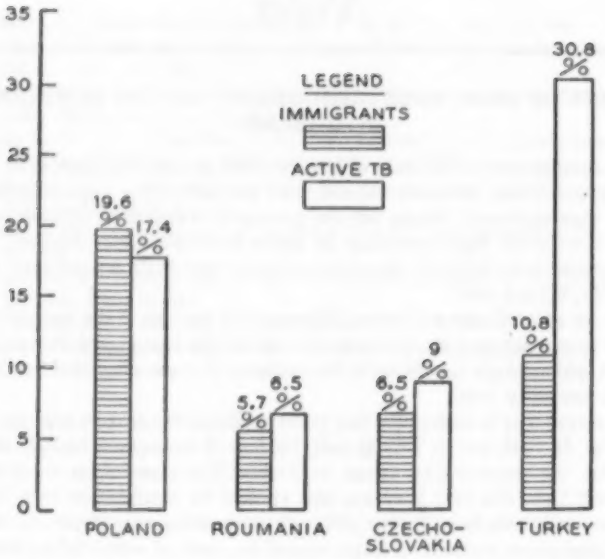

FIG. 1. Immigration and morbidity in 1949 according to country of origin.

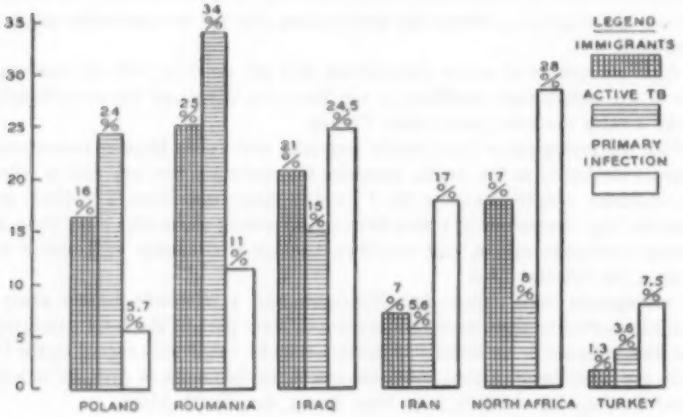

FIG. 2. Immigration and morbidity in 1950 according to country of origin.

morbidity rate among European Jews and a lower one among Orientals. The proportion was reversed in regard to childhood tuberculosis.

In order to compare the results of mass roentgenography in the reception camp in Israel with those in the United States (where those examined roentgenographically represent a stable population) in addition to the clinical classification, i.e., *active, questionably active, inactive* tuberculosis, the classification in use internationally was introduced: *minimal, moderately advanced, far advanced* lesion.

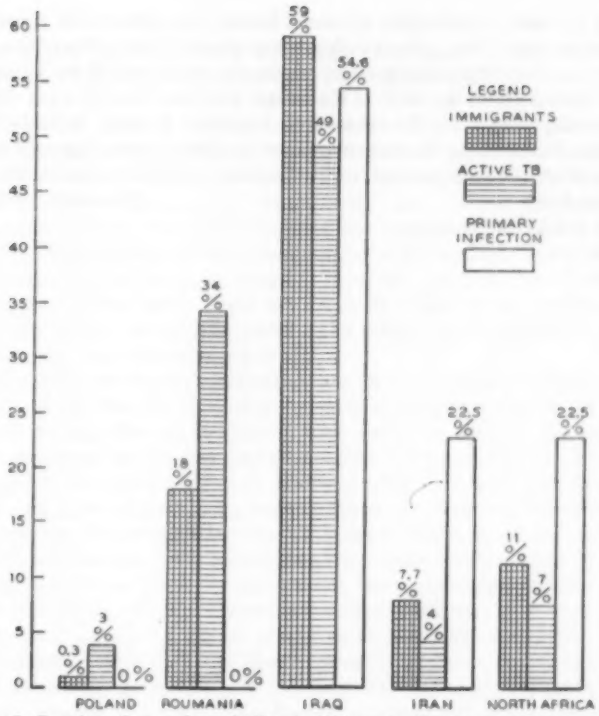

FIG. 3. Immigration and morbidity in 1951 according to country of origin.

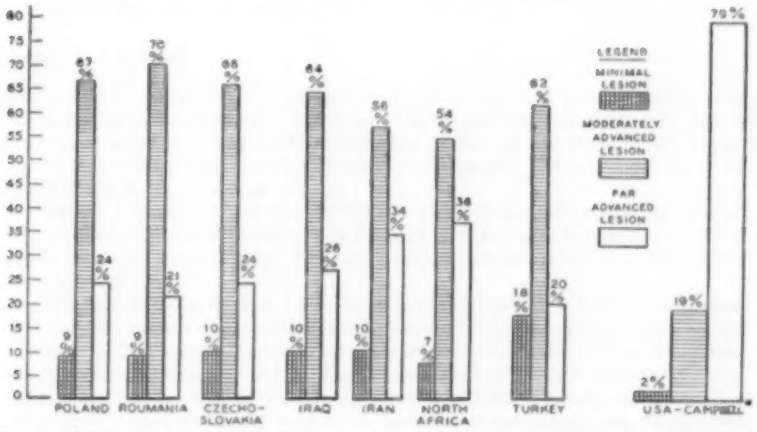

FIG. 4. Tuberculosis morbidity in accordance with international classification in 1949–1951.

* Campbell, A. J.: New York State J. Med., 1949, *49*.

A majority of cases of moderately advanced lesions were observed in Israel, while in the United States most of the patients suffered from minimal lesions. Far advanced tuberculosis occurred in 7 to 10 per cent in Israel (among the immigrants from Turkey, 18 per cent). In the United States this form of the disease was found in only 2 per cent.

It is noteworthy that, while the tuberculosis morbidity is lower in Arabic countries than in Europe, various lung diseases uncommon in Europe were diagnosed among the Orientals in a relatively large number, e.g., echinococci, cystic diseases, et cetera.

Workers Sick Fund — Hermann Lichtenstein
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July 6, 1953

THE USE OF QUARTZ DUST FOR CHALLENGING THE VIABILITY OF TUBERCLE BACILLI IN TUBERCULOUS LESIONS

Viability of the tubercle bacillus in tuberculous lesions constitutes an important problem in assaying the effectiveness of an antimicrobial agent used for the treatment of tuberculosis. The problem has provoked much discussion, especially since the advent of surgical resection of the so-called "target point lesion." Many divergent views have been expressed. Usually the debate involves the basic question which was concisely stated by Dr. Gladys Hobby at the annual meeting of the National Tuberculosis Association in Los Angeles, California, on May 18, 1953. Her question was: "If a tuberculous lesion contains acid-fast bacilli which are visible on smear but no organisms can be recovered by cultivation on artificial media or by guinea pig inoculation, is one justified in concluding that the organisms are dead?"

Since early 1953, the Saranac Laboratory has been employing a method which is presented here in order that it may be tried by other investigators working on the problem. The method involves the use of finely divided crystalline silica in the form of quartz dust. It is predicated on the fact that quartz dust stimulates infection by the tubercle bacillus, even of attenuated strains such as Rl and BCG which have little, if any, capacity to produce progressive disease in the healthy guinea pig. Evidence indicates that quartz dust produces its adverse effect by creating a tissue milieu, which is favorable for the growth and multiplication of the tubercle bacillus, rather than by enhancing the inherent virulence of the bacillus. Thus, the method may have special application for the propagation of bacilli which, although present in a tuberculous lesion, may have an extremely low vitality and possibly an altered growth requirement because of the antimicrobial therapy. It is conceivable that such bacilli are easily overcome by the native tissue resistance of the healthy guinea pig and, therefore, they fail to grow when inoculated into that animal. However, such bacilli, even those in a state of suspended animation and with a potential infectibility, may multiply and produce disease in tissue when that resistance is lowered by the action of quartz.

Materials and Methods

Quartz dust: The quartz dust is a commercial product specially treated in order to obtain particles in a range 3 microns and less in size. Size is important since it has been shown that particles larger than 3 microns have relatively little biologic effect on tissue; particles in the ultramicroscopic range are still being investigated. The technique for obtaining particles of the proper size is as follows:

Grinding: A batch of approximately 500 gm. of freshly ground quartz dust and 250 ml. of distilled water is placed in a ball mill of one liter capacity with a number of balls, either porcelain or agate. The mill is rotated mechanically for approximately five hundred hours.

Sedimentation: Separation of the particles according to size is based upon Stokes' law for sedimentation of spherical particles. Since the quartz particles are irregular in shape, sedimentation is not strictly in accordance with that law. Therefore, based upon past experience with quartz dust, the procedure is to allow sedimentation for four hours, then to draw off the uppermost layer of fluid to a depth of 3.5 cm. It is that layer of fluid which will contain the particles of the desired size. The process can be repeated by restoring to the original batch the amount of water removed, re-agitating the mixture with a stirring rod and again allowing sedimentation to

occur. Obviously, there is a limit to the number of separations which may profitably be carried out because the 3.5 cm. layer of water will become gradually clearer, depending upon the number of particles, 3 microns and less in size, still remaining in the sediment. Usually the yield of those particles is unprofitable beyond repeated separation exceeding fifteen to twenty times. The separated fluid which has been drawn off is then evaporated, leaving a residue of dry quartz dust.

Aqueous suspension of the quartz dust: Ten grams of the dried quartz dust are suspended in 100 ml. of distilled water in the following manner: The dust is placed in a large watch glass. Small amounts of the water are added gradually to the edge of the mass from which portions are stirred to produce a fluid paste. Proper stirring is accomplished by using the end of a glass rod covered by a short length of rubber tubing. The paste is transferred to a flask and the total volume is brought to 100 ml. The suspension is sterilized by autoclaving for fifteen minutes at 20 pounds' pressure and subsequently it is stored in the refrigerator until used.

Tuberculous material: With sterile precautions, the tissue is sectioned so that the cavity contents are exposed and other tuberculous areas may be noted. Smears made directly from the material are stained for acid-fast organisms. Varying amounts of the caseous or necrotic material, preferably all of it, are removed from the cavity or other areas and are suspended in 2 to 3 ml. of sterile normal saline.

Nodular and other tuberculous lesions are ground in a mortar and pestle with a small amount of saline. Coarse sand may be added. It is preferable, however, to use a mechanical juicer which will assure complete maceration of the tissue. The resulting specimen is allowed to stand for five to ten minutes in order to permit sedimentation of the coarse substance and to obtain a relatively clear supernatant.

The suspension is thoroughly mixed either with a spade or sterile glass rod and 1 ml. is pipetted into each of two test tubes. One milliliter of saline is added to one tube and 1 ml. of the 10 per cent quartz suspension is introduced into the other tube. After agitation, a measured quantity from each tube is inoculated onto egg-yolk-potato-flour medium (ATS).

Guinea pig inoculation: Two healthy guinea pigs are used, one receiving the suspension without quartz and the other the suspension with quartz. One milliliter is injected subcutaneously into the flank of each pig. One month after inoculation, the pigs are tested for skin hypersensitivity to an intracutaneous dose of 0.1 ml. of 3 per cent of Old Tuberculin. If the skin test is negative at that time, additional tests are made at monthly intervals. An animal giving a positive skin test at one month is killed two weeks later; the mate-test pig is also killed for comparison. An animal showing a positive skin test at two months is killed at that time as is also its mate-test pig. Animals with negative tests are killed at three months. Smears and cultures are made of tissue from all animals and questionable lesions are studied histologically.

To date, the method has been applied to a small number of tuberculous lesions removed surgically from patients under chemotherapy. The results look promising, as, in one series of nine lesions which contained acid-fast bacilli by smear, five of the nine gave positive results in guinea pigs which had received the respective suspension to which the quartz dust had been added; but the results were negative in those pigs inoculated with the suspension without quartz dust. In another series of five lesions in which acid-fast bacilli were not discovered by smear, two of the five gave positive results when quartz dust was added to the suspension; but the same lesions were negative when tested with the suspension without quartz.

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December 31, 1953

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Correspondence

TREATMENT OF A RECENT TUBERCULIN REACTOR

To the Editor of the American Review of Tuberculosis:

In the November, 1953, issue of the REVIEW, pages 819–836, is published a report of the panel discussion on *Present Concepts of Antimicrobial Therapy in Pulmonary Tuberculosis*, held on May 19, 1953, at the Los Angeles meeting of the Ameridan Trudeau Society. Though not a member of the panel, I was asked a question by the Chairman (yourself) and my reply was apparently not clearly recorded, since it is misquoted in the report (page 834). The error, I believe, resulted because I did not walk to a microphone but merely arose from my chair in the audience. Since the subject is important, may I take this opportunity of correcting the statement?

The question concerned the treatment of a person who had recently become a tuberculin reactor but had no other evidence of tuberculosis. Specifically, you asked whether I would treat a hypothetical 22-year-old nurse who had recently become a tuberculin reactor. My reply, as reported, is that "I would treat her, not with three drugs, but with one, just as Bill (Tucker) said." In fact, my reply was that I would *not* treat her, and I went on to ask Dr. Tucker whether he would and, if so, whether with one or with several drugs. (Dr. Tucker had previously indicated he would treat a *child* who had recently become a tuberculin reactor with drug therapy, but had not specified the drug or drugs he would select.)

Having thus corrected the record, I should like to add that I would still answer the question in the same way. Perhaps it might stimulate discussion in your columns to elaborate and state my reasons.

The reasons I would not favor treating an adult, or any other person save possibly an infant, with no other evidence of tuberculosis than a tuberculin reaction, even though known to be recently acquired, are as follows:

1. No drugs, administered singly or concurrently, which are presently approved and generally available are known to be eradicative for *M. tuberculosis* in humans.

2. Consequently, there is little reason to think that treatment of tuberculous infection in humans, even at this early stage, will surely prevent the possible later development of progressive tuberculosis.

3. The risks from any of the available drug therapies are not inconsiderable, and the possible remote toxic sequelae are as yet not fully known.

4. The risk of serious tuberculosis eventually developing, without becoming detectable while successful treatment is still possible, although significant, is not great in any but the youngest age group, i.e., infants.

In the case of infants who have become reactors spontaneously, i.e., through exposure to natural infection and not by vaccination, I believe that treatment with isoniazid should be considered, since the incidence of miliary tuberculosis and tuberculous meningitis as an immediate sequel of primary infection is high in this age group. The evidence, first noted by ourselves[1], that isoniazid is highly effective in the prevention of meningitis, even in the presence of generalized miliary disease, has been confirmed from many sources. Since meningitis is still the most serious of all forms of tuberculosis, there is a very good case, therefore, for prophylactic therapy in this age group in which the incidence, in terms of tuberculin reactors, is very high. As there is little evidence that streptomycin (or

[1] CLARK, C. M., ELMENDORF, D. F., CAWTHON, W. U., MUSCHENHEIM, C., and MCDERMOTT, W.: Am. Rev. Tubere., 1952, *66*, 391.

streptomycin and PAS) is of comparable protective value, I would not select such therapy as a substitute for isoniazid. Neither is there solid evidence that the addition of streptomycin, PAS, or both would add materially to the value of isoniazid in this circumstance. Moreover, since streptomycin and PAS are more difficult to administer, and apparently more toxic, this is an additional reason not to employ these drugs for this particular purpose.

Very truly yours,
CARL MUSCHENHEIM

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NEW YORK, NEW YORK
February 26, 1954

PNEUMOTHORAX INDUCTION

To the Editor of The American Review of Tuberculosis:

The editorial by Dr. Constantine Vysniauskas in the January, 1954, issue of the REVIEW seems to me to be largely a one-sided expression of opinion with respect to the mechanism of pneumothorax induction. There are not many who published experiments designed to investigate this problem and, therefore, it is not proper to say "that the majority of investigators consider the accidental pneumothorax as a preventable complication, not as a rule."

Although I do not agree with Tchertkoff and his associates that one should *deliberately* attempt to puncture the lung when inducing a pneumothorax, it is quite reasonable to assume that most inductions are traumatic. My experience with a small series of cases handled as in the experiment of Tchertkoff *et al.* has convinced me of this (J. Phila. Gen. Hosp., 1952, *3*, 22). It is difficult to imagine that a needle of any sort does not puncture both the visceral and parietal pleura at the time of induction, no matter how dexterous the operator.

Of course, in most cases, the laceration of the visceral pleura is undoubtedly small and rapidly closes over so that relatively little air escapes from the lung. Consequently, the small amount of air released from the lung may not be noticeable, especially if air is injected by the operator. The fact that an air leak from the lung is not demonstrated by our crude roentgenographic methods in the hands of some people is no proof that the lung is not ordinarily lacerated at induction. Certainly, the sudden aspiration of fluid from the syringe in the technique described by the writer of the editorial is no proof that the lung has not been punctured, for one may reason that as soon as the visceral pleura is punctured, air escapes from the parenchyma, the lung begins to collapse, fluid from the syringe is sucked into the pleural cavity, and a negative intrapleural pressure can be registered on the manometer as the lung falls away from the point of the needle. Thus, the fact that Dr. Vysniauskas does not pull back the needle in order to obtain manometric readings does not rule out lung puncture as the mechanism responsible for the initial registration of negative pressures.

Dr. Vysniauskas neglects to mention the work of Ornstein *et al.* (Quart. Bull., Sea View Hosp., 1946, *8*, 5), who actually measured the volume of gas present in the pleural cavity after induction and found it greater than the volume of air injected. It would be difficult to explain these findings without postulating laceration of the lung. It is true that, with a careful technique, traumatic pneumothorax large enough to be of clinical significance does not often occur. However, there is good evidence to support the con-

tention that most, if not all, inductions of pneumothorax occur through puncture of the lung and there is as yet no good proof to the contrary.

17959 Redwood Road
Castro Valley
California

Sincerely,
William Weiss

January 16, 1954

ISONIAZID DELIRIUM

To the Editor of The American Review of Tuberculosis:

W. H. C., an Englishman, at age 35, first came under my care on August 3, 1930, his tuberculosis having been diagnosed in January, 1929, when he had far advanced disease with cavity on each side. He also had evidence of tuberculosis in the ileocecal region. He did very well, indeed, both cavities on the right and the one of the left having closed when he left here in 1932. He returned here on a number of occasions for examinations until 1944, during which time the disease showed steady calcification.

He said that he had not been perfectly well since a viral infection of December, 1945. He had bronchopneumonia in December, 1951, which recurred in March, 1952, and again in December, 1952. Relapse of his tuberculosis was established in March, 1953, in Philadelphia. Streptomycin-PAS treatment was begun on March 16, 1953, and he returned to Saranac Lake quite sick on May 18, 1953. He had developed consolidation with atelectasis of the right upper lobe and bronchopneumonic infiltration in the left middle third. A positive culture of May 19, 1953, showed a 1-plus growth in 10 units of streptomycin and 20 colonies of 0.1 per cent PAS.

He felt very sick and had a very severe cough. Slight hemoptysis occurred on June 25, lasting several days. He had a marked inspiratory wheeze on the right side suggestive of bronchial stenosis.

When the report of resistance to streptomycin was obtained on July 9, 1953, I recommended 100 mg. of isoniazid three times daily. This was immediately followed by some very bizarre symptoms. On the very first day of this drug treatment, he felt "cuckoo," dizzy. He could not coordinate in sight, thought, or action. For instance, he noticed that while he was reading he would be reading words ahead of the place on which his attention was. Vision was slightly blurred. Sometimes when he would reach for a glass of water on the bedside table he would miss it by several inches. He wanted to talk to people; he talked a lot to himself, a thing he never had done before. He felt "as if he were outside of his body looking down on himself"; walked in his sleep. On one occasion the nurse found him in the bathroom and he did not know that he was there. On the whole, though, he felt that he could think rationally. He was very restless, he felt stimulated; there were choking sensations. He was not depressed but he did think that he was going out of his mind. These symptoms did not increase on the second and third day of treatment, at the end of which time the treatment was stopped. The following day he felt normal.

On August 29, without much hope of success, I suggested to him that we might resume the isoniazid, starting with a low dose and increasing to a full dose. He began, therefore, with 25 mg. increasing by 25 mg. every day to 150 mg. so that by the end of September he was taking 300 mg. a day without any reaction. This drug was given while continuing to take streptomycin, 1 gm. daily, and PAS, 12 gm. daily. A splendid symptomatic improvement has followed the use of the isoniazid.

This patient had no apprehension about taking isoniazid when it was first prescribed. He had no idea of having any bad reaction to it. He seemed quite stable, emotionally, before he began the use of the drug.

The reaction he had to isoniazid resembled that which was seen in some patients who had been taking iproniazid. I made absolutely sure that he had received isoniazid. The fact that he could take the drug when given the second time in ascending doses will probably be interesting to others who see any peculiar reactions following the use of this drug.

19 Academy Street
Saranac Lake, New York

Sincerely yours,
J. W. Hayes

November 25, 1953

RESISTANCE OF TUBERCULIN REACTOR

To the Editor of The American Review of Tuberculosis:

Two communications (Palmer, C. E., and Shaw, L. W.: Am. Rev. Tuberc., 1953, *68*, 462; Dubos, R. J.: Am. Rev. Tuberc., 1953, *68*, 472) in the September, 1953, issue of The American Review of Tuberculosis provide contrasting data on the development of tuberculosis in tuberculin-positive and tuberculin-negative groups. The report of Palmer and Shaw indicates that, in Muscogee County, Georgia, tuberculin reactors are approximately three times more likely to develop tuberculosis than nonreactors. In German concentration camps, as noted in Dubos' discussion of a book by seven Danish physicians, tuberculin-negative Danish prisoners developed pulmonary or extrapulmonary tuberculosis approximately three times more often than those who had been tuberculin-positive.

The essential difference between these two epidemiological situations is that in the concentration camps both reactors and nonreactors were equally exposed to infection, whereas in Georgia the two groups were not equally exposed. The former case is similar to the general hospital, where the danger of infection is shared by all student nurses; the latter case is typical of community exposure, which is not shared by all segments of the population: thus, persons residing in wealthier and less crowded suburbs do not experience the same risk of infection as the residents of a slum district or the members of a tuberculous family.

In the concentration camps, the tuberculin reactors presumably developed disease less often because (*a*) a certain enhancement of resistance is usually associated with tuberculin allergy, (*b*) in the face of heavy exposure (the tuberculosis mortality was 30 to 40 per cent of the total mortality at some camps) the factor of endogenous tuberculosis is relatively minor, and (*c*) those tuberculin-positive but roentgenographically negative before deportation from Denmark represented a selected group, since those in whom primary tuberculosis infection caused disease were not included in these observations.

In the Georgia study, the infected and uninfected persons did not undergo the same risk of exposure. Obviously, if the nonreactors were never exposed, they could never develop tuberculosis. The tuberculin reactors in these circumstances more often developed tuberculosis because of (*a*) occasional endogenous development of the disease and (*b*) the very fact of having been infected prior to entering the study reflects residence in a household or neighborhood where exposure is more likely to continue or recur.

Similar considerations were involved in a study made several years ago at the Henry Phipps Institute of the relationship of childhood infections to the development of tu-

berculosis in adult life. There can be no straightforward answer to the question "Is the tuberculin reactor more resistant to or more susceptible to tuberculosis?" The answer, as is evident from the conflicting findings in many investigations, differs in different epidemiological circumstances.

GRADUATE HOSPITAL
UNIVERSITY OF PENNSYLVANIA
PHILADELPHIA 46, PENNSYLVANIA

Sincerely yours,
HAROLD L. ISRAEL

December 23, 1953

IRREGULAR DISCHARGE IN THE U. S. A. AND GREAT BRITAIN

To the Editor of The American Review of Tuberculosis:

I note with interest Dr. George Day's letter published in your Correspondence section in the October, 1953, issue of THE AMERICAN REVIEW OF TUBERCULOSIS, dealing with the self-discharge problem. I feel the differences between the higher self-discharge figures in this country and the lower ones in England are due to other factors than those referred to in his communication. I do not believe the nature of the educational material presented to patients in written form is crucial to this problem. In so far as educational material is a part of more comprehensive patient services, this statement may require modification; but whether the publications are self-help in tone or otherwise, there is no evidence to indicate that this has any appreciable effect on the self-discharge rate.

Moreover, it seems that the American tendency to play down the death theme is in concordance with the reality of diminishing death and increasing hope. I do not feel there is an overly euphemistic or overly morbid attitude toward patients in this country. Dr. Day feels we are urging our patients to "stop being lepers." I do not know where this idea comes from; it is true that some patients, in their psychological reactions, feel like lepers, but the entire attitude of acceptance of the disease and the diminishing stigmata attached to it has not, so far as I know, brought forth any visible evidence that we are exhorting our patients to stop being lepers. The attitudes toward educational material, death, and contagion vary widely over our large country, and often from doctor to doctor in any single sanatorium. It would be interesting to have a careful study of prevailing professional attitudes.

If a patient keenly feels the stigmata of his disease, it avails little to advise him to stop feeling that way; and so far as I know, in many sanatoriums in America psychiatrists and physicians trained in psychological medicine are trying to work with the feelings of patients which lead to such reactions. Certainly, the idea of being a "leper" in the historical conscience belongs more to England than to America.

In regard to the differences between the two cultures, it seems to me that the problem of the self-discharge patient falls into four classifications:

1. Socio-economic: Concerning this, it is worth pointing out that in 1950 there were 11,000 patients in England awaiting admission to 23,000 beds. The privilege of having a bed and the realization that if one left it one might not again find one's way into the sanatorium must be a strong deterrent to leaving the hospital. The patient's reaction to so-called reality problems are of equal importance, the most obvious of which (in the breadwinner) is the availability of economic assistance. To judge from personal communications, this is much more favorably met in England than in the United States.

By and large, it appears to this American observer (who has visited England on several occasions) that the British are a much less restless people, and for centuries they have

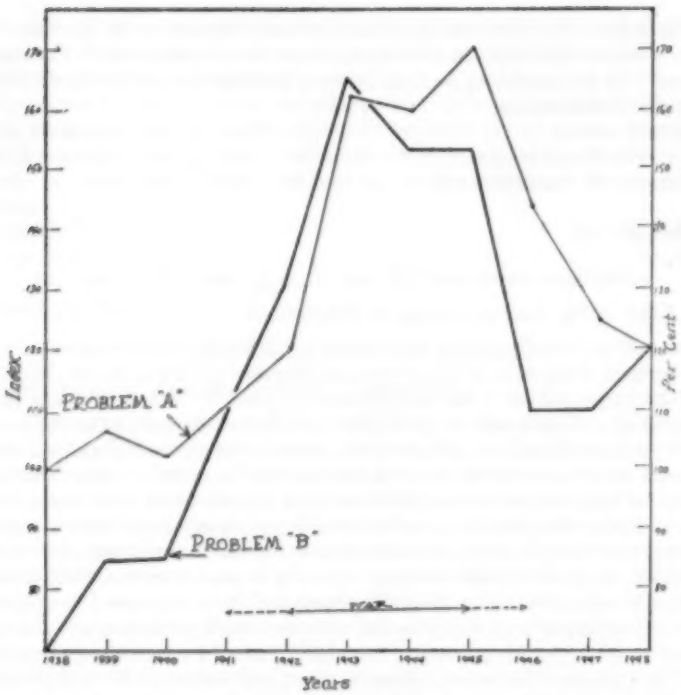


FIG. 1. "A": Showing curves of juvenile court cases reported at Mid-Century White House Conference on Children and Youth. "B": Showing per cent of irregular discharge in a large multiple-county California sanatorium.

been developing an adaptation to an island culture with concomitant limitation of movement. I have dealt with acculturation in a previous paper ("A Hitherto Undescribed Island," American Scientist, July, 1953, *41*, 456).

Another example of factors profoundly affecting the self-discharge rate is the times of unrest and national crisis: that is to say, crises in personal life often have their stimulus in crises in the social environment. In figure 1 this point is illustrated. Without changing the index or per cent of two graphs I happened to see within a week of each other, I superimposed them and found a remarkable similarity. Problem "A" is the juvenile court cases as reported at the Mid-Century White House Conference on Children and Youth, in which the year 1938 is taken as 100. Problem "B" is the irregular discharge, expressed in per cent, from a large multiple-county California sanatorium, with an average irregular discharge of 125 per year. The average regular discharge at this santaorium was 264. Both of these graphs show that disturbances in family life and major shifts in community living have a fairly direct *relationship to behavior*. Either children are charged with delinquency or patients leave the sanatorium against medical advice. The causes may be deep-seated personality problems, but viewed in the aggregate the magnitude of the problem seems clearly to follow the same course in relationship to World War II: a rapid rise with pre-war tensions, a peak early in the war, a decline in mid-war, and a much more rapid post-war decline than the pre-war rise.

In the immediate post-war year of 1945, the irregular discharge rate in the Veterans Administration hospitals was 72.5 per cent; by 1947, it was 54.4 per cent.

2. The second large problem is that of interpersonal relationships, which have their effect on the morale of the sanatorium. Industry for some time has known the disrupting and demoralising effect of a poorly fitted supervisor; it knows that work productivity varies with workers' satisfaction. The same is true in the sanatorium and takes place between patient and patient, patient and staff, patient and family. Of course, the adjustment to interpersonal tensions has something to do with the culture of the sanatorium, i.e., private, Veterans Administration (all males), charity, and so forth, and it has something to do with the culture and tradition of the country. The British, whose magnificent tradition of stoicism and determination, characterized by their traditional greeting, "Carry on,"—a people who almost unwincingly endured the horrors of the Battle of Britain, whose relationship with civil authority is such that their police do not carry firearms—these and many other factors could be cited to illustrate cultural differences in attitude toward acceptance. Factors such as these seem to me of far greater importance than the American attitude toward infectivity and contagion, the masks, and the aseptic technique. I do not know what relationship this has to the self-discharge rate; no studies on it have appeared. It is a matter of conjecture, which relates to Point 3.

3. It is important to recognize that there is a variability from sanatorium to sanatorium and from year to year and, while the over-all self-discharge rate in the United States is much too high, many sanatoriums are meeting the problem quite adequately. It is to be hoped that more sanatoriums with low self-discharge rates will report on their experience and will attempt to study in scientific fashion their favorable results. Perhaps more will be learned from them than from the unhappy situation in other sanatoriums.

Figure 2 and table 1 show the self-discharge rates from 22 published papers, ranked according to magnitude of the problem and arbitrarily divided into quartiles. For purposes of description, these break down with the discharge over 50 per cent as *alarming*, over one-third as *critical*, over 25 per cent as *serious*, and below this as *favorable*. These, however, are generalizations, particularly in the *favorable* group, for while 20 per cent might be a favorable figure for a large city hospital, 5 per cent might be favorable for a small county sanatorium.

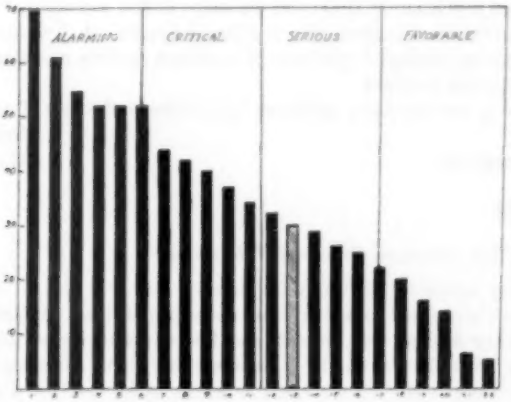


FIG. 2. Twenty-two published self-discharge reports ranked according to per cent of self-discharge and divided into quartiles.

TABLE 1

SANATORIUM OR GROUP	SELF-DISCHARGE RATE	YEAR	AUTHOR AND COMMENTS
1. National Sanatorium (Tenn.)	71	1924	Klotz (all males)
2. 4,190 Patients (Wisc.)	61	1924–34	Wisc. St. Bd. of Voc. Educ.
3. VA hospitals	54.4	1946–47	Tollen (all males)
4. Glendale (U. S. A.)	52	1947	Weiss
5. New York metropolitan sanatorium	52	1949	Drolet and Porter
6. Philadelphia General Hospital	52	1940	Epstein and Hetherington
7. 3 Pennsylvania state sanatoriums	44	1944	Collected study
8. 13 New Jersey sanatoriums	41.5	1948	Brewster
9. Iowa state sanatorium	40	1938	Galinsky and Brownstein
10. 17 sanatoriums in 16 states	32	1933	Witney and Dempsey
11. 14 state and county sanatoriums (N. J.)	34	1941	Frankel
12. Firland Sanatorium (Wash.)	37	1952	Northrup, Fountain & Zahn
13. San Francisco city and county hospital	30	1952	Wilmer
14. All New York metropolitan sanatoriums	29	1945	Tubere. Conf. Metrop. N. Y.
15. Wisconsin state and private sanatoriums	26	1945	Filek
16. 13,825 patients in sanatoriums (U. S. A.)	25	1945	Drolet and Bobrowitz
17. Licking County Sanatorium (Ohio)	22	1941	Johnson (5-year period)
18. Baltimore (Md.) City Hospitals	20	1951	Beacham
19. Colindale (England)	16	1938–51	Snell (all males)
20. Middle River Sanatorium (Wisc.)	14	1932	Carpenter
21. San Mateo County Sanatorium (Calif.)	6.5	1950	Wilmer
22. Pinewood (England)	5	1947	Fowler (all females)

4. Intrapersonal problems: It is beyond the scope of this communication to deal with the area where, in the final analysis, the problem is fought out. I would only point out that both the deep psychological problems of a patient and his reactions to staff, sanatorium, and disease are involved.

These, as I see it, are the major problems that influence the rate of self-discharge.

510 Byron
Palo Alto, California

Sincerely yours,
Harry A. Wilmer

November 9, 1953

To the Editor of The American Review of Tuberculosis:

Dr. George Day replies to Dr. Harry A. Wilmer:

Some time ago I was asked to review three phonographic discs entitled, "Adjusting to Tuberculosis." They seemed very elegant, and I was just taking up my pen to say so, when I thought, "Well, how about it? I know nothing about adjusting to tuberculosis. But some of my patients do. I'll get them to review it for me." So I played the discs over our Internal Broadcasting System to all headphones and asked listeners to write

down their comments and let me have them. Their response was magnificent, and I learned quite a lot. My eyes were opened for the very first time to the vast differences of approach to tuberculosis (both on the part of the patients and doctors) that existed on either side of the Atlantic; which was really the theme of my letter in your October issue. Recordings which had evidently been a smash hit in the U. S. A. reduced my British patients to states of bewilderment, spluttering indignation, or helpless laughter according to temperament.

A major outburst occurred within the first two minutes of playing time. The Sanatorium audience is asked, "Why are you here?" and is answered, "It is because you have a contagious disease." With one voice my patients protested, "I was not sent here because I was suffering from a contagious disease. I came here because sanatorium treatment is more convenient and more certain than treatment at home. I am not a leper, and I don't like being told I'm a leper." So you see, please Sir, it wasn't I, it was my patients who misconstrued your End of Treatment as Stopping Lepers being Lepers.

My patients were puzzled, moreover, by a very lengthy plea that they should not discharge themselves against medical advice. The average response was, "Why on earth should I? If I put myself in the hands of a doctor I follow his advice. I presume he knows best." (Another transaltantic difference?)

The upshot of all this was that, at the instigation of my patients and with their help, guidance, and stern censorship, I knocked out a British version of, "Adjusting to Tuberculosis." Any reader who can conscientiously find nothing better to do may locate it in *Tubercle*, March, 1953. . . . It appears to find favor with British patients, but it may very well send American patients and doctors screaming up the wall. If it does—and I rather hope it does—it will substantiate my contention, i.e., that we view tuberculosis somewhat differently.

That's all.

Sincerely,

GEORGE DAY

THE SANATORIUM
MUNDESLEY
NORFOLK, ENGLAND

January 26, 1954

Books

LIVING WITH A DISABILITY. By Howard A. Rusk and Eugene Taylor, in collaboration with Muriel Zimmerman and Julia Judson. Pp. 282, The Blakiston Company, Garden City, New York, 1953, Price, $3.50.

Intended for the person with a physical disability, this book graphically and expertly summarizes numerous self-help devices which aid in the performance of the daily activities of life and of remunerative employment. By its calm, helpful approach it should further stimulate the disabled to lead a less dependent and more satisfactory life. The book is straightforward and practical with no maudlin sentimentality. It will serve well to further assist the disabled person to live more efficiently and easily with his handicap, no matter how great the magnitude.

Beginning with the basic performances of eating, drinking, and elimination, which may be severely affected by physical disability, the authors describe and picture many self-help devices which can be bought or constructed to make life more independent. A wider sphere of personal usefulness, more happiness, and greater individual comfort result.

Despite the wide variety of helpful devices described and pictured which were gathered from medical libraries, hospital clinics, rehabilitation centers, current periodicals, from that treasury of Americana—the mail order catalogues, and from individuals, the pertinent advice given to the disabled is to do without special equipment if possible. If not, then a device may be tried. Every self-aid pictured or described has been tested clinically and found to be practical. The book overflows with well-chosen, clear, convincing photographs and simple helpful sketches of an extraordinary variety of self-aids. It is stressed that not all of the devices listed or procedures outlined are of equal value or importance; nor is it inferred that any handicapped person would be wise to obtain every item set forth as usable by someone with a disability similar to his own.

The chapters on "Looking Your Best" and "Keeping Up Appearances" are delicately handled; yet the challenge to the handicapped is a stimulating one. The importance of not using an overt physical disability as an excuse for not maintaining personal appearance is stressed. Convincing photographs of disabled persons using numerous self-aids also document this portion of the book.

For the estimated 10,000,000 handicapped women in this country, the chapter on the "Conquest of the Kitchen" is a boon. In fact, it can be studied with profit by all housewives and not merely by the handicapped homemaker. Although specific diseases are not mentioned, it is obvious that the housewife with a chronic pulmonary disease could easily learn how to lighten her task and conserve her strength by reading this section. Even a glance at the numerous photographs will show how a disabled woman can increase her physical scope in the kitchen.

For those fortunate enough to build their own homes to meet the problems of their specific handicaps, the chapter "Living With Your Home" provides many excellent ways to make the home environment less formidable. Certainly no individual or responsible group should attempt the construction of a home for a handicapped person without careful study of this portion of the book.

It is apparent that this book deserves widespread distribution.

KENNETH T. BIRD
Waltham, Massachusetts

BOOKS RECEIVED

ELEVEN BLUE MEN AND OTHER NARRATIVES OF MEDICAL DETECTION. By Berton Roueche. Pp. 215, Little, Brown & Company, Boston, 1954, Price, $3.50.

ACUTE PULMONARY EDEMA. By Mark D. Altschule. Pp. 68, Grune & Stratton, New York, 1954, Price, $3.50.

ADVANCES IN THE CONTROL OF ZOONOSES. BOVINE TUBERCULOSIS, BRUCELLOSIS—LEPTOSPIROSIS, Q FEVER—RABIES. WHO/FAO Seminar on Zoonoses, Vienna, November, 1952. Pp. 275, World Health Organization, Palais des Nations, Geneva, 1953, Price, $3.00.

APORTACION DE LA ESCUELA ESPANOLA A LA TECNICA ENDOBRONQUIAL. BY S. Garcia-Vicente. Pp. 43, Paseo de la Castellana, 76, Madrid, 1953.

LOS ANGELES COUNTY-WIDE CHEST X-RAY SURVEY OF 1950. A Statistical Analysis and Review, Primarily of Tuberculosis Findings. By Godias J. Drolet. Tuberculosis Control Foundation, Los Angeles, 1953.

American Trudeau Society

Medical Section of the National Tuberculosis Association

Informal Report on Veterans Administration Thirteenth Conference on Chemotherapy of Tuberculosis

Dr. Arthur M. Walker was especially requested to allow the REVIEW *to publish this informal recital of the proceedings of the recent Veterans Administration Thirteenth Conference on the Chemotherapy of Tuberculosis, held in St. Louis early in February of this year.*

The Transactions *of the Conference will eventually be available. As is so frequently the case, however, there will be a lamentable gap between the actual deliberations of the Conference and the dissemination of the information therein presented. It is hoped that the publication of Dr. Walker's informal memorandum at this time will serve as a step in narrowing the gap.*—The Editor.

The Thirteenth Conference has come and gone. Although, because of its number, we viewed its approach with some concern, it was sufficiently successful so that we have no hesitation in advising the taller hotels to renumber their upper floors. The papers were good, the weather Floridian, the hotel comfortable, the auditorium of the St. Louis Medical Society, where the scientific sessions were held, as spacious and comfortable as our memories of 1952 recalled it to be. The primary purpose of this memorandum is to lay the reports of the several committees in your hands at the earliest moment (it will be a matter of months before the *Transactions* can be published). But we will introduce them by some informal account of the meetings for the benefit of the men who were not there. This account, depending as it does upon the notes of a single individual, will almost certainly be inaccurate as well as improperly weighted, and the responsible reader is advised to possess his mind with patience while waiting upon publication of the *Transactions*.

Quantitative analysis: The registration numbered 320 doctors, a figure considerably in excess of previous conferences and of which, incidentally, only 70 were financed through the Veterans Administration. This progressive increase in attendance (at the First Conference, in 1946, 34 persons sat in a tight semicircle for three days gazing devotedly at X-ray view-boxes) has necessarily been attended by an increase in formality although we have tried very hard to preserve some of the "one-big-family" atmosphere. In the present instance, it must be admitted that the auditorium presented more or less the appearance, *sine voce*, of a Republican (Democratic, if you wish) Convention; there was a vast collection of recording apparatus with four guardian angels, and wandering microphones were dashed up and down the aisles whenever anyone in the audience had what he thought to be a bright idea needing expression. Fifty-four papers were presented at the five scientific sessions but sufficient restraint was exerted by the speakers, and sufficient skill by the chairmen, so that the schedule was closely adhered to without the use of alarm clocks or "red and green lights," to quote from Herb Mantz. It should be added that at least 50 per cent of the lantern slides were readable; although this is not too impressive a figure, it exceeds our previous record (and, parenthetically, that of some other meetings) and, non-parenthetically, we despair of increasing it further by the written word, since it must be admitted that a number of people are congenitally unable to realize that the human eye can only read a limited number of figures in a limited number of seconds.

The routine, which has been developed over many years, was again followed: February

8, 9, and the morning of February 10, were devoted to presentation of scientific papers; the afternoon of February 10, to the meeting of committees; and the morning of February 11, to the presentation of reports by these committees.

As a mark, one supposes, of our advancing age, only one formal evening meeting was held. As you have already been informed, the expenses of this conference were provided, in the absence of VA funds, by an Anonymous Donor (caps by intent) and the recording equipment by another friend. It was held, in a very genuine sense, with the cooperation of the National Tuberculosis Association. Not only did they agree to handle the funds involved but also, by holding the meetings of their Policy Committee and those of the American Trudeau Society's Executive Committee and Committee on Therapy during the two days preceding the conference, they added sensibly to the quality and quantity of the attendance. In point of fact, it became difficult to meet anyone whom one did not know.

Qualitative analysis: By way of general comment, it may be remarked that spot checks showed attendance did not fall below 290 and that the well-known Dr. X, an attendant at many meetings and a confirmed corridor conversationalist, only missed listening to one paper in two days.

Ascending now to the particular: The consolidated tables, which are of course the solid core of the study, were less referred to than ever before; they consisted, on this occasion, of 79 pages in exceedingly fine type and have really become the province of a student in his study rather than of a page-turning audience; their content was summarized in lantern slides hastily prepared for the occasion but, another year, this would preferably be presented by the statisticians who compiled them and in even briefer form—assuming that these two aims are sympathetic. It should be emphasized that there is vastly more material obtainable from the punch cards than it has been technically possible to reproduce in the tables.

To become more particular: No breath-taking advance in either understanding or therapy was reported. Indeed, to have had two such advances (streptomycin and isoniazid) within ten years is more than one could reasonably expect. The several drugs which were brought to the attention of the conference, e.g., variations upon the theme of PAS, analogues of isoniazid, substituted thioureas, a chemical combination of streptomycin with isoniazid, and the isonicotinyl hydrazide of glucuronalactone, did not appear sufficiently promising to warrant incorporation in the new protocols for the cooperative study. Paradoxically enough, the chief interest was aroused by a drug, pyrazinamide, which, given in combination with isoniazid, displayed impressive therapeutic effects both in mice and man; and, *in vitro*, markedly delayed the development of resistance to isoniazid. The drug had almost been abandoned at the previous (Twelfth) Conference but is now to be studied in an extensive pilot study, with due regard to the hepatotoxicity reported by one of two investigators, if a supply can be obtained. It makes one wonder how many other drugs we have discarded in the past because they were only tried (as was the case in this instance) upon patients who had received previous chemotherapy and whose tubercle bacilli were already resistant to streptomycin. The other "newsworthy" item was the fate of bacteriologically proved miliary and meningeal tuberculosis treated by a combination of streptomycin, PAS, and isoniazid (without intrathecal therapy). The survival rate, which had previously been notably increased by the addition of PAS to streptomycin, was further increased (to approximate 90 per cent) by the triple combination in 89 cases; *no* patients developed meningitis following miliary disease and, although the series has only been observed for eighteen months, past experience has indicated that a majority of deaths occur during this period. This is, then, highly encouraging. The use of intrathecal

streptokinase, streptodornase, and PPD was reported ineffective in children with tuberculous meningitis.

The conference was opened by a paper emphasizing our experience (we shall purposely avoid mentioning the names of authors) with the importance of concurrent controls, of which we have conceived a high opinion. A discussion of the background factors, e.g., such matters as age, sex, extent, and trend of disease, reported for the various regimens displayed such a considerable uniformity as to give confidence to the randomization procedures that are being employed but should not, perhaps, be discussed at such length in the future unless egregious discrepancies display themselves. The series of streptomycin-PAS regimens which were embarked upon in January, 1951, had now come to a reasonable maturity and, totaling 1,500 cases of pulmonary tuberculosis, merely confirmed the more tentative conclusion of last year that very little could be said to the advantage or disadvantage of streptomycin given twice weekly or daily in doses of 1.0 or 0.5 gm. in so far as objective measurements upon X-ray change, cavity closure, and sputum conversion were concerned.

The more recent "basic" regimens, adopted in August, 1952, following a preliminary pilot study of streptomycin-isoniazid, streptomycin-PAS, and isoniazid-PAS, were in too early a stage and too few in number ("cohort" is the word used by our statisticians) to deserve serious consideration; but, again, so far as that consideration goes, their results were very similar. Indeed, perhaps the most striking thing about these studies is that they are not striking, and one is left hanging upon theoretical arguments in making one's choice between them. The United States Public Health Service described the present status of their experience with various combinations of the three drugs. One cannot but be impressed with the similarity of planning indulged in by them, by the Medical Research Council of Great Britain, and by ourselves, despite the lack of any coordination between the three groups; one would be tempted to ascribe it to mental telepathy—in part transatlantic—but the easier solution is that the processes of thought involved are of a relatively low caliber. Fitzsimons Army Hospital reported relatively unsatisfactory results with the triple combination of streptomycin-PAS-isoniazid but, quite aside from the theoretical objections to this regimen, one could not but remark that the comparison was made with regimens which had been employed in earlier years.

It seems to us that the most important pilot studies which have been undertaken in recent years by the VA-Army-Navy group were those dealing with the optimal duration of prolonged chemotherapy, pneumoperitoneum, pulmonary resection, and rest therapy, which were started last spring. It was, of course, too early to anticipate any results from these, but they will be a source of anticipation for the next conference.

The incidence of radiologic relapse following completion of chemotherapy would appear to be of the order of 5 per cent in patients treated with streptomycin-PAS; the persistence of cavitation appeared to affect this percentage unfavorably, i.e., to increase it. The favorable effect of prolonging chemotherapy was not yet decisive, and other factors which were analyzed, e.g., age, were without apparent effect upon it. In the case of patients still receiving chemotherapy, progression of disease has been definitely associated with the development of resistance to streptomycin; this is an observation of some years' standing. Since the introduction of isoniazid, the exciting claim has been made that the development of resistance to *this* drug was not accompanied by progression and, furthermore, that the isoniazid-resistant tubercle bacilli were not infective to experimental animals. Evidence was presented to the conference that both of these claims were, at best, half-truths. Neither positive nor negative evidence could be adduced for the thought (it has never been a "claim") that these bacilli were noninfective for man, nor does it seem likely to appear for some years.

The session which was billed under the heading of "Laboratory" produced the usual number of interesting papers and the usual amount of disagreement, but will be touched on very lightly here. The demonstration that urinary PAS produces a red color when mixed with calcium hypochlorite is of practical importance. Isoniazid is without effect upon either the EEG or psychometric tests; the peripheral neuritis which it is known to produce is apparently due to an effect upon pyridoxine metabolism which can be avoided by the administration of pyridoxine or (for some reason not clear to the present writer) vitamin B12. Preliminary studies indicate that an isoniazid-resistant mutant of the Vallée strain has similar immunizing properties to BCG in guinea pigs. The final paper was a brilliant discussion by Dr. Woodward of Baltimore (here, we break our rule) on the effect of chemotherapy upon immune processes in nontuberculous disease.

The surgical session, sans benefit of the tables, was told that the consolidated data showed a further decrease in the incidence of complications following pulmonary resections and the use of pyrazinamide, either alone or in combination with PAS or isoniazid, came in for special praise from one hospital. That hardy perennial question, the viability of tubercle bacilli in resected lesions (both open and closed), was the subject of a series of papers and considerable discussion. As far as the writer could determine, the situation was but little changed from that of last year: Those who formerly believed that the bacilli (liberally visible on smear but neither susceptible to culture nor capable of producing infection in animals) were dead now believed that they were deader than ever; whereas those who believed that they were merely in a resting state continued to criticize the bacteriologists for inability to awaken them and drew an apt analogy to the leprosy bacillus. The latter school of thought was favored by one paper in which (a new culture medium being employed) viable, i.e., reproducing, bacilli were recovered in 67 per cent of 35 closed necrotic lesions; others reported only 17 to 34 per cent of positive cultures. The question is, obviously, of enormous importance since upon its answer depends the indication (or lack of indication) for resection. It is devoutly to be hoped that the pilot study presently in progress will eventually permit a decision in this matter. The number of genitourinary cases available to the study now totals 675, of which 75 per cent are renal; the percentage of urine conversions is holding up remarkably well after three years' experience with streptomycin-PAS, and a trial of streptomycin-PAS-isoniazid is now under way.

It is proposed to hold the 14th Conference in Atlanta, Georgia, during the early part of February, 1955.

NOTES

COURSE IN MEDICAL MYCOLOGY

A course in Medical Mycology has been offered continuously during the summer for the past seven years at Duke University School of Medicine and Duke Hospital. The course will be offered again this summer and has been designed to give the student a working knowledge of the human pathogenic fungi and an understanding of the diseases which they cause.

Instruction by members of the departments of medicine, pathology, and bacteriology will emphasize the clinical, pathologic, and therapeutic aspects of fungus infections. Patients, clinical materials, cultures, and laboratory animals will be available for study. An opportunity to study pathologic materials, gross and microscopic, will be given those whose interest and previous training would make this of value to them. The practical laboratory aids which help to establish a definitive diagnosis will be stressed.

The course is open to clinicians, pathologists, bacteriologists, technicians, and others who have an interest in the medical phases of Mycology. Classes meet six days a week from July 5 to July 31.

Inquiries concerning this course should be directed to Dr. Norman F. Conant, Duke Hospital, Durham, North Carolina.

$750,000 GIVEN BY MARKLE FUND TO AID 25 SCHOLARS

The John and Mary R. Markle Foundation announced yesterday the appointment of twenty-five Scholars in Medical Science, all faculty members of medical schools in the United States and Canada, the largest number to be appointed in any year since the Scholar program began in 1948. The fund has appropriated $750,000 toward the support of these doctors and their research, to be granted at the rate of $6,000 annually to the twenty-five medical schools where they will teach and carry on their research. John M. Russell, executive director of the foundation, said that a total of $3,950,000 has been appropriated toward the support of 136 doctors in 59 medical schools during the seven years the program has been in operation.

The purpose of the program is to offer both academic security and financial help to faculty members at the start of their careers in academic medicine. The twenty-five Scholars were selected from sixty candidates nominated for the grants by deans of medical schools, each of whom presented a five-year program for the candidate indicating the school's plans "for his progress up the academic ladder," according to Mr. Russell.

INSTITUTE ON ALCOHOLISM FOR REGISTERED PROFESSIONAL NURSES

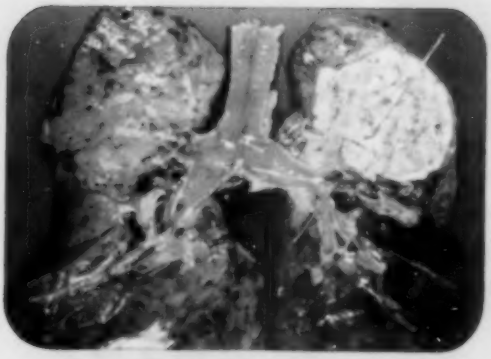
An Institute on Alcoholism for registered professional nurses will be held at Yale University from July 29 through July 31, 1954. The sessions are being planned to acquaint a limited number of nurses with the nature and extent of alcoholism and with theories concerning the etiology of addiction.

Attention will be directed toward the effectiveness of various treatment resources. A particular emphasis will be given to the role of the nurse in interpreting to patients or to their families the nature of alcoholism and the necessity of securing specialized assistance following hospital treatment for acute intoxication.

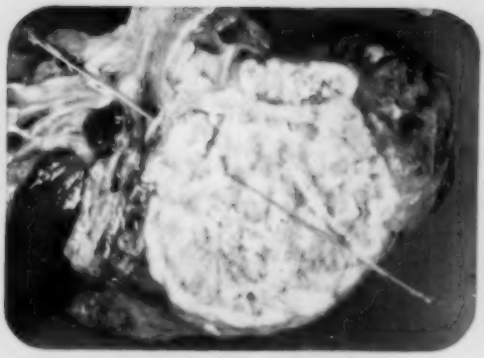
The course will consist of lectures and discussion sessions with a staff drawn from the faculties of Yale University and other institutions.

This Institute is sponsored by the Yale University School of Nursing, the Summer School of Alcohol Studies, and the Connecticut League for Nursing.

Inquiries should be addressed to: Yale Center of Alcohol Studies, 52 Hillhouse Avenue, Yale Station, New Haven, Connecticut.

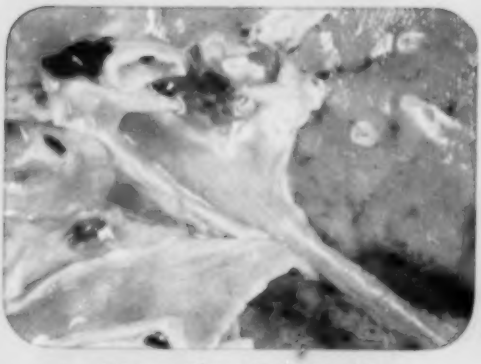


Bronchogenic Carcinoma.



Bronchogenic Carcinoma (close-up).

Multiple Lung Abscesses—Septic Aneurysm.



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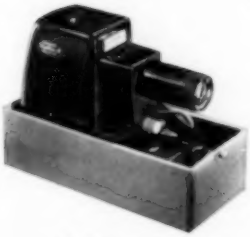

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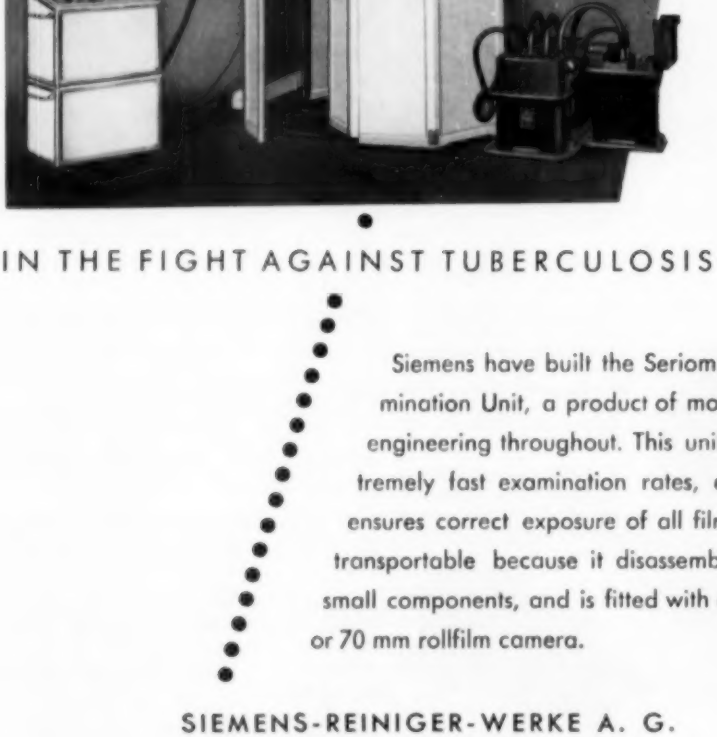

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Robitzek, E., and Ellerbrook, M.: Streptomycin and Oxytetracycline-Para (Terramycin) in the Treatment of Pulmonary Tuberculosis, Am. Rev. Tuberc. 67:18 (Jan.) 1953.

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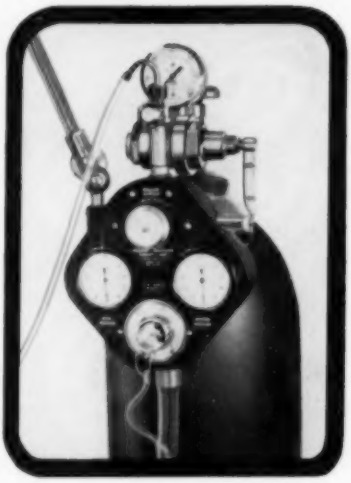

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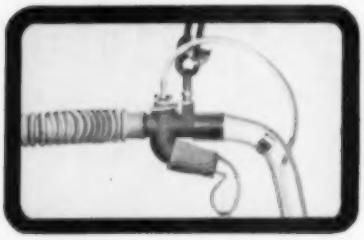

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1. Hobby, G. L., Lenert, T. F., Rivoire, Z. C., Donikian, M., and Pikula, D.: Am. Rev. Tuberc. 67:808, 1953.

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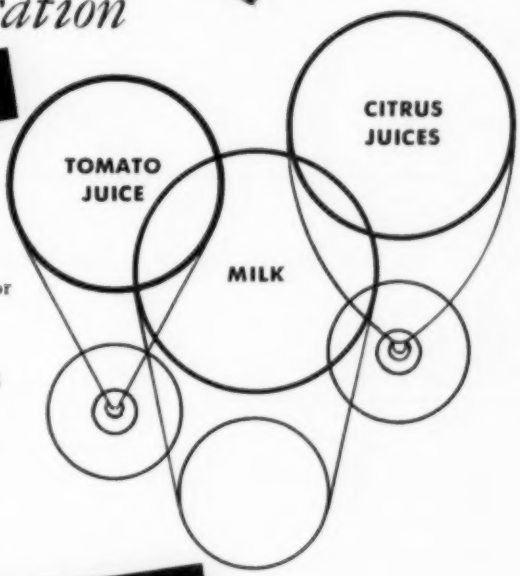

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